SECTION 2.6.1—INTRODUCTION

LENACAPAVIR, SUCUTANEOUS INJECTION

Gilead Sciences

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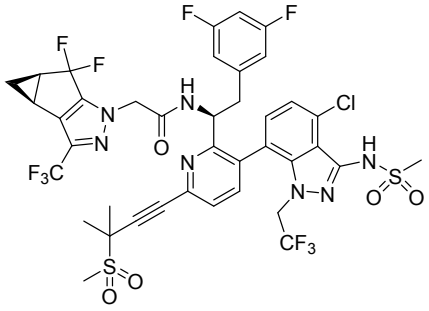
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1. NONCLINICAL SUMMARY

Lenacapavir is a novel, first-in-class, multistage, selective inhibitor of HIV-1 capsid function being developed for the treatment of HIV-1 infection.

Lenacapavir has a molecular formula of $C_{39}H_{32}ClF_{10}N_7O_5S_2$. The free acid has a molecular weight of 968.3 Dalton (1µM = 0.968 µg/mL)

Figure 1. Chemical Structure



Lenacapavir inhibits HIV at multiple points in the viral lifecycle, including interfering with capsid-mediated nuclear uptake of preintegration complexes and impairing virion production and proper capsid core formation. Virus produced in the presence of LEN display aberrantly shaped capsids. These malformed virus particles can still infect a new target cell but cannot replicate as they are unable to support reverse transcription without a properly formed capsid core.

A comprehensive program of nonclinical studies has been conducted for lenacapavir; summaries of the nonclinical data are provided in m2.6. and the completed nonclinical study reports are provided in m4 and m5.

The nonclinical data presented in this dossier support the favorable benefit/risk profile for the proposed use of lenacapavir for the treatment of HIV-1 infection. All information from nonclinical studies that is relevant to the prescriber and patient has been included in the proposed prescribing information and patient labeling.

SECTION 2.6.2—PHARMACOLOGY WRITTEN SUMMARY

LENACAPAVIR

Gilead Sciences Inc.,

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LIST OF ABBREVIATIONS

Alu-LTR	junction of Alu restriction enzyme recognition sequence and LTR sequence
ATV	atazanavir
AZT	azidothymidine
CA	capsid
CC_{50}	cytotoxic concentration giving 50% killing of the cells
CCR5	C-C chemokine receptor type 5
C_{max}	clinical maximum plasma concentration
C_{min}	clinical plasma trough concentration
CNS	central nervous system
CPSF6	cleavage and polyadenylation specificity factor subunit 6
CRF	circulating recombinant form
D4T	stavudine
DMSO	dimethyl sulfoxide
DRV	darunavir
DTG	dolutegravir
Early-RT	early reverse transcription product
EC_{50}	effective concentration giving 50% inhibition of virus replication
EC_{95}	effective concentration giving 95% inhibition of virus replication
ECG	electrocardiogram
EFV	efavirenz
ELISA	enzyme-linked immunosorbent assay
EQD	equilibrium dialysis
EVG	elvitegravir
FIH	First-In-Human
FTC	emtricitabine
Gal-PC-3	galactose-adapted PC-3 cells
Gal-HepG2	galactose-adapted HepG2 cells
GCSM	Gag cleavage site mutation
GLP	Good Laboratory Practices
GS-6207	GS-714207, also known as lenacapavir (LEN)
HBV	hepatitis B virus
HCV	hepatitis C virus
hERG	human Ether-à-go-go Related Gene ion channel
HIV	human immunodeficiency virus
HRV-16	human rhinovirus serotype 16
IN	integrase
INSTI	integrase strand transfer inhibitor
IQ	inhibitory quotient
ITMN-191	HCV protease inhibitor from InterMune

JET	jacketed external telemetry
K_D	equilibrium dissociation constant
kg	kilogram
Late-RT	late reverse transcription product
LEN	Lenacapavir, also known as GS-6207 or GS-714207
LTR	long terminal repeat
mg	milligram
MI	maturation inhibitor
mL	milliliter
µM	micromolar
MOI	multiplicity of infection
nM	nanomolar
NNRTI	non-nucleotide reverse transcriptase inhibitor
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NRTI	nucleoside reverse transcriptase inhibitor
NTCP	sodium taurochlorate cotransporting polypeptide
NUP153	nucleoporin 153
NUP358	nucleoporin 358
NVP	nevirapine
p24	capsid
p55	Gag polyprotein
p160	Gag-Pol polyprotein
$paEC_{95}$	plasma protein binding-adjusted EC_{95}
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PI	protease inhibitor
PR	protease
RAL	raltegravir
RBV	ribavirin
RPV	rilpivirine
RSV	respiratory syncytial virus
RT	reverse transcriptase
SC	subcutaneous
SD	standard deviation
SPR	surface plasmon resonance
TAF	tenofovir alafenamide
TAM	thymidine associated mutation
TDF	tenofovir disoproxil fumarate
TFV	tenofovir
T_{max}	time to reach the maximum concentration (C_{max})

WT	wild-type
YM-53403	RSV polymerase inhibitor from Yamanouchi Pharmaceuticals

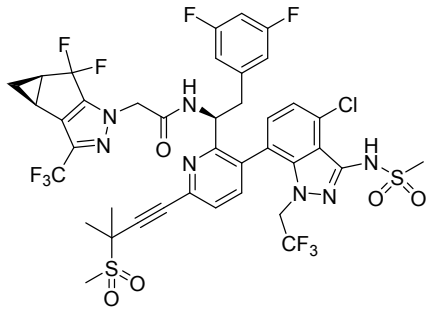
Abbreviations are in accordance with those accepted by Toxicology and Applied Pharmacology.

1. BRIEF SUMMARY

The viral capsid (CA) protein plays multiple essential roles in the HIV-1 replication cycle, including the assembly and maturation of new virions, the encapsidation of the viral RNA genome and replicative enzymes into a protective conical capsid shell, the coordination of reverse transcription of the viral genome into DNA following virus entry, directed cytoplasmic trafficking of capsids to the nucleus along microtubules, docking and active nuclear import of intact capsids via nuclear pore complexes, and controlled nuclear disassembly of capsids to enable the release of their encased viral DNA near sites of integration {Burdick 2020, Zila 2021}. Mature CA (p24) protein consists of two α-helical domains connected by a flexible linker and is formed after proteolytic cleavage of the viral Gag (p55) and Gag-Pol (p160) precursor polyproteins by the viral protease. The CA protein then spontaneously assembles in maturing virions into higher-order oligomers to form a conical lattice comprising ~ 250 CA hexamers and 12 CA pentamers {Ganser 1999}. Proper assembly and disassembly of this capsid shell is essential for HIV-1 particle infectivity {Freed 2015, Sundquist 2012}.

Lenacapavir (LEN, GS-6207) is a potent, selective, multi-stage inhibitor of HIV-1 capsid function and is proposed as a novel therapeutic for the treatment of HIV-1 infection (Figure 1).

Figure 1. Structure of Lenacapavir



Protein X-ray crystallography and biochemical assays indicate that LEN binds with high affinity and specificity to recombinant HIV-1 CA protein at the intermolecular interface between the N-terminal domain of one subunit and the C-terminal domain of the neighboring subunit within the same CA hexamer. Lenacapavir binding to CA protein increases the rate and extent of in vitro CA assembly, resulting in malformed capsid polymers when visualized by electron microscopy {Link 2020}.

In acutely infected cells, LEN binding to capsid results in potent suppression of both early- and late-stage processes essential for HIV-1 replication, with EC_{50} values of 0.023 nM and 0.439 nM, respectively. This dual mechanism of action is unique among all clinically used antiretroviral classes. Lenacapavir interferes with late-stage virus production and capsid core formation events. Lenacapavir also interferes with a capsid-mediated early-stage process occurring after reverse transcription but before the integration of viral DNA. Lenacapavir reduces the accumulation of integration products and abortive two-long-terminal-repeat (2-LTR)-containing circles, both of

which form exclusively in the nucleus {Bukrinsky 1993}. Lenacapavir interacts with capsid at a binding site shared by multiple host proteins implicated in the active transport of HIV-1 capsids into the nuclear compartment, including cleavage and polyadenylation specificity factor 6 (CPSF6), nucleoporin 153 (NUP153) and nucleoporin 358 (NUP358) {Bhattacharya 2014, Matreyek 2013, Price 2012, Price 2014}. Thus, LEN binding is expected to prevent these host cell factors from binding to HIV-1 CA protein and thereby interfere with the active nuclear import of capsids and their encased viral pre-integration complexes, an essential step in the formation of an integrated provirus.

Lenacapavir is a potent and selective inhibitor of HIV-1 replication in the MT-4 T-lymphoblastoid cell line, in primary human $CD4^+$ T lymphocytes, and in monocyte-derived macrophages, with EC_{50} and selectivity index (CC_{50}/EC_{50}) values ranging from 0.030 to 0.190 nM and 140,000 to >1,670,000, respectively. Lenacapavir also displays potent antiviral activity against two HIV-2 isolates, but its potency is 15- to 25-fold lower relative to its potency against HIV-1.

Lenacapavir maintains full antiviral activity against a broad spectrum of HIV-1 mutants and HIV-1 clinical isolates resistant to currently approved antiretrovirals from the nucleotide and nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), integrase strand transfer inhibitor (INSTI) and protease inhibitor (PI) classes.

Lenacapavir also maintains full antiviral activity against patient-derived HIV-1 isolates containing naturally-occurring Gag polymorphisms, in contrast to compounds from the maturation inhibitor (MI) class which displayed strongly reduced susceptibility in the presence of most naturally-occurring Gag polymorphisms. This observation confirms that LEN and MIs exert their antiviral activity through different mechanisms.

The antiviral activity of LEN is not affected by the presence of Gag cleavage site mutations associated with PI treatment or MI resistance, further underlining the lack of cross-resistance between existing drug classes and LEN.

Lenacapavir selects for single and double CA amino acid substitutions, including L56I, M66I, Q67H, K70N, N74D/S and T107N. HIV-1 variants encoding these individual mutations confer resistance to LEN, with the majority of these capsid HIV-1 mutants, including those conferring moderate to high-level LEN resistance, displaying substantially reduced replication capacity relative to the WT virus in $CD4^+$ T-lymphocytes and macrophages. CA population-level and deep sequence analyses suggest that all known LEN resistance associated mutations are not present above 1% of the patient population.

Lenacapavir-resistant mutants remain fully sensitive to drugs from other classes (NNRTIs, NRTIs, PIs, INSTIs, MIs).

Lenacapavir displayed similar antiviral activity across clinical isolates from all major HIV-1 subtypes with a mean EC_{50} value of 0.05 nM (ranging from 0.02 to 0.16 nM) in PBMCs, and a mean EC_{50} value of 0.24 nM (ranging from 0.15 to 0.36 nM) in the HEK293T cell line. Consistent with these findings, the CA protein sequence is highly conserved across 8 major HIV-1 subtypes, including the majority of residues within the LEN binding site. Most capsid polymorphisms near the LEN binding site displayed WT susceptibility to LEN.

Lenacapavir demonstrated synergistic in vitro antiviral activity when combined with antiretrovirals from other classes, including TAF, EFV, DTG and DRV, respectively representative of NRTI, NNRTI, INSTI, and PI classes.

Lenacapavir selectively inhibits HIV-1 with no measurable antiviral activity against hepatitis C virus (HCV) and human rhinovirus (HRV) and only weak antiviral activity against hepatitis B virus (HBV) and respiratory syncytial virus (RSV). Lenacapavir is at least 29,000-fold more active against HIV-1 as compared to HBV and RSV and thus it is not expected to exert any clinical activity against these viruses at drug exposures relevant for the inhibition of HIV-1.

Lenacapavir showed low cytotoxicity in primary human hepatocytes (CC_{50} > 50 μM) and in several non-target human cell lines of different tissue origin (CC_{50} values > 44 μM), including two cell lines that were adapted for growth in galactose-containing medium to increase their sensitivity to mitochondrial toxicity.

Lenacapavir showed low potential for off-target effects when tested against a panel of 87 molecular targets at a concentration of 10 μM, at least 5,000-fold above the estimated free drug concentration at the clinical maximum plasma concentration (C_{max}) after the oral loading and subcutaneous dosing regimen (6-month dosing).

In a single SC dose safety pharmacology study in rats, LEN was associated with a minor non-statistically significant increase in tidal volume and a decrease in respiration rate at 100 mg/kg . No LEN-related changes in tidal volume or respiration rate were noted in animals administered ≤ 30 mg/kg up to 72 hours postdose, and there were no LEN-related changes in minute volume in animals administered ≤ 100 mg/kg. No LEN-related changes in behavioral/central nervous system (CNS) endpoints were observed in the rat 4-dose repeat dose toxicology study that dosed up to 100 mg/kg. In dogs, no electrocardiogram (ECG) or blood pressure changes were attributed to LEN after a single dose of 100 mg/kg or after 4 SC doses of 30 mg/kg. The free C_{max} at the no-observed-(adverse)-effect levels (NO(A)ELs) for respiratory, CNS and cardiovascular endpoints were 1.4, 1.4 and 20-fold higher than the free C_{max} after the 6-month clinical oral loading and subcutaneous dosing regimen.

In summary, LEN is a potent, selective and multi-stage inhibitor of HIV-1 capsid function, a mechanism that is orthogonal and complementary to existing anti-HIV therapies.

2. PRIMARY PHARMACODYNAMICS

2.1. Antiviral Activity

The inhibition of HIV-1 replication in vitro by LEN was evaluated in the MT-4 T-lymphoblastoid cell line, in primary human $CD4^+$ T-lymphocytes, and in monocyte-derived macrophages. The antiviral activity of LEN was also tested against HIV-1 clinical isolates in human peripheral blood mononuclear cells (PBMCs), as well as in HEK293T cells (Monogram Biosciences assay). The effect of multiplicity-of-infection (MOI) on the antiviral activity of LEN was also studied in a human T-cell line.

2.1.1. Activity of LEN in the MT-4 T-Cell Line

The antiviral activity of LEN was evaluated using a 5-day cytopathic assay in the MT-4 T-cell line acutely infected with HIV-1 (IIIB strain) (m2.6.3, Section 2, PC-200-2018, Table 1). Lenacapavir displayed potent antiviral activity in MT-4 cells, with an effective concentration to achieve 50% inhibition (EC_{50}) value of 0.19 nM. In addition, the effective concentration to achieve 95% inhibition (EC_{95}) of LEN in MT-4 cells was calculated from the EC_{50} and Hill slope values that were determined from a high resolution 40-point dose response curve. Lenacapavir showed a Hill slope of 3.5 in MT-4 cells, yielding an EC_{95} value of 0.23 nM. Taking into account the human serum shift determined by equilibrium dialysis (m2.6.5, Section 4, AD-200-2020), LEN exhibited a plasma protein binding-adjusted EC_{95} ($paEC_{95}$) value of 4 nM, which was used for the estimation of clinical inhibitory quotient (IQ) for the projected trough concentration of LEN in humans.

Table 1. EC_{50}, Hill Slope and EC_{95} of LEN in MT-4 Cells

	MT-4 T-Cell Line					
	Standard Resolution	High Resolution				
Compound[1]	EC_{50} (nM)[2]	EC_{50} (nM)[3]	Hill Slope[3]	EC_{95} (nM)	EQD Shift (Fold Change)[4]	$paEC_{95}$ (nM)
LEN	0.19 ± 0.05	0.10 ± 0.01	3.51 ± 0.31	0.23 ± 0.02	17.4	4.0 ± 0.4
EFV	1.38 ± 0.64	0.79 ± 0.06	3.25 ± 0.59	2.0 ± 0.15	22.4	44 ± 3
DTG	1.92 ± 0.90	1.34 ± 0.14	2.14 ± 0.28	5.3 ± 0.55	29.5 ± 11.2	156 ± 16
ATV	10.7 ± 3.4	7.23 ± 0.50	3.13 ± 0.15	18.5 ± 1.3	8.1	150 ± 11

1 EFV = efavirenz; DTG = dolutegravir; ATV = atazanavir
2 Standard resolution EC_{50} values represent the geomean (± SD) from at least 19 independent experiments (Data from PC-200-2018).
3 High resolution EC_{50} and Hill Slope values represent the mean (± SD) from at least 2 experiments (Data from PC-200-2018).
4 Equilibrium dialysis (EQD) shift values were obtained from a single run for EFV, or represent the mean values obtained from 5 runs for DTG and 2 runs for LEN and ATV (Data from AD-200-2020).

2.1.2. Activity of LEN in Primary Human CD4+ T-Lymphocytes and Macrophages

The antiviral activity of LEN was determined in human primary CD4+ T-lymphocytes and monocyte-derived macrophages acutely infected with HIV-1 (BaL strain) using 7-day and 12-day virus production assays, respectively (m2.6.3, Section 2, PC-200-2019). Lenacapavir displayed potent anti-HIV-1 activity in each of these physiological target cells for HIV-1 replication, with EC_{50} values of 0.06 nM and 0.03 nM for $CD4^+$ T-lymphocytes and monocyte-derived macrophages, respectively (Table 2).

Table 2. Activity of LEN in Primary Human Target Cells

Compound[1]	EC_{50} (nM)[2]	
	$CD4^+$ T-Lymphocytes	Monocyte-Derived Macrophages
LEN	0.06 ± 0.02	0.03 ± 0.01
EFV	1.23 ± 1.1	0.29 ± 0.22
DTG	0.96 ± 0.38	1.87 ± 0.31
ATV	6.88 ± 5.69	8.31 ± 4.99

1 EFV = efavirenz; DTG = dolutegravir; ATV = atazanavir
2 EC_{50} values represent the mean (± SD) obtained from 3 independent donors (Data from PC-200-2019).

2.1.3. Activity of LEN Against HIV Clinical Isolates in Human PBMCs

The antiretroviral activity of LEN was tested against 23 clinical isolates of HIV-1 and two isolates of HIV-2 in freshly isolated human PBMCs (m2.6.3, Section 2, PC-200-2020). Lenacapavir displayed potent antiviral activity against all tested HIV-1 clinical isolates representing all major subtypes with a mean EC_{50} value of 0.05 nM and a narrow range of antiviral potencies with EC_{50} ranging from 0.02 – 0.16 nM (Table 3). In comparison, DTG exhibited mean and median EC_{50} values against the same tested HIV-1 isolates of 0.70 and 0.67 nM, respectively. Lenacapavir also showed potent antiviral activity against two HIV-2 isolates but was 15- to 25-fold less active relative to HIV-1 isolates. These results indicate the potential for LEN to be effective clinically against a broad range of HIV-1 strains.

Table 3. Summary of LEN Activity Against HIV-1 Clinical Isolates

Compound[1]	EC_{50} (nM)[2]		
	Mean ± SD	Median	Range
LEN	0.05 ± 0.03	0.04	0.02 – 0.16
DTG	0.70 ± 0.26	0.67	0.38 – 1.18
AZT	18.24 ± 13.73	13.20	1.32– 51.60

1 DTG = dolutegravir; AZT = azidothymidine
2 Total: n = 23; Subtypes A, B, C, D, E, F, G, CRF01_AE, CRF02_AG: n= 2, 8, 2, 2, 2, 1, 1, 1, and 2, respectively; Group N and O: n=1 each. Individual EC_{50} values represent the mean of triplicate measurements in human PBMCs (Data from PC-200-2020).

2.1.4. Activity of LEN Against Diverse HIV-1 Clinical Isolates in HEK293T Cells

The antiretroviral activity of LEN was tested in vitro against 40 clinical isolates of HIV-1 representing diverse subtypes and including 3 isolates harboring HIV protease inhibitor (PI) resistance mutations (m2.6.3, Section 2, PC-200-2041). For the 37 WT, Lenacapavir IC_{50} values against tested isolates ranged from 0.148 to 0.357 nM, with a mean of 0.238 nM. In comparison, the mean IC_{50} values for the HIV protease inhibitors atazanavir (ATV), darunavir (DRV), and lopinavir (LPV) against the same tested isolates were 7.87 nM (range: 2.47 – 14.5), 0.947 nM (range: 0.209 – 2.74), and 11.4 nM (range: 2.60 – 35.1), respectively (Table 4). Overall, LEN showed potent antiviral activity against all HIV-1 isolates evaluated, regardless of subtype or presence of drug resistance mutations.

Table 4. Antiviral Potency of LEN and Control Compounds Against Wild-Type HIV-1 Clinical Isolates

	IC_{50}, nM (n = 37)	
Compound	**Mean***	**Range**
LEN	0.238	0.148 - 0.357
ATV	7.87	2.47 - 14.5
DRV	0.947	0.209 - 2.74
LPV	11.4	2.60 - 35.1

ATV = atazanavir; DRV = darunavir; LEN = lenacapavir; LPV = lopinavir; (*) geometric mean (Data from PC-200-2041)

2.1.5. Activity of LEN at Different MOIs

The in vitro antiviral activity of LEN was determined against HIV-1 (NL4-3 strain) and compared with TAF, EFV, DTG, and DRV in a human T-cell line infected with a wide range of MOIs (m2.6.3, Section 2, PC-200-2031). Lenacapavir demonstrated potent antiviral activity at all tested MOIs (MOI ranged from 0.01 – 1.25), with EC_{50} values ranging from 0.028 nM to 0.455 nM (16-fold increase) across the 125-fold range in virus inputs (Table 5). Whereas the in vitro antiviral efficacy for DRV varied by less than 3-fold across the same range of MOIs, the EC_{50} values for TAF, EFV, and DTG ranged from 10 nM to 61 nM (6.1-fold increase), 0.69 nM to 6.26 nM (9.1-fold increase), and 0.31 nM to 2.46 nM (7.9-fold increase), respectively. Thus, LEN is a potent inhibitor of WT HIV-1 and, similar to other antiretroviral drugs, the in vitro antiviral activity of LEN is affected by the level of infection. At the highest tested MOI, the EC_{50} value of LEN was 5- to >100-fold lower than any of the tested representative inhibitors from other antiretroviral classes.

Table 5. Activity of LEN in a Human T-Cell Line at Different MOIs

HIV-1 Infection Condition	EC_{50} (nM)[1]				
MOI	**LEN**	**TAF**	**EFV**	**DTG**	**DRV**
0.01	0.028 ± 0.013	10.0 ± 4.5	0.69 ± 0.30	0.31 ± 0.13	1.36 ± 1.46
0.05	0.050 ± 0.016	12.8 ± 7.9	1.15 ± 0.34	0.50 ± 0.22	1.33 ± 0.70
0.25	0.152 ± 0.074	50.5 ± 48.5	2.79 ± 1.73	1.57 ± 1.10	2.67 ± 2.42
1.25	0.455 ± 0.168	61.1 ± 25.0	6.26 ± 1.22	2.46 ± 1.01	3.84 ± 1.60

DRV = darunavir; DTG = dolutegravir; EFV = efavirenz; MOI = multiplicity of infection; TAF = tenofovir alafenamide;
1 EC_{50} values represent the mean (± SD) obtained from 4 independent experiments (Data from PC-200-2031).

2.2. Mechanism of Action

The interaction of LEN with HIV-1 CA protein was defined through X-ray crystallography and biophysical characterization of the compound binding affinity and effects on in vitro CA assembly {Link 2020}. The in vitro antiviral activity of LEN was determined against distinct stages of the HIV-1 replication cycle in the MT-2 T-cell line. The mechanism(s) by which LEN inhibits late-stage replication events was investigated by evaluating the effect of the compound on virus capsid core morphology using electron microscopy and on virus production and maturation using p24 ELISA and Western blot analysis of Gag cleavage products. The mechanism(s) by which LEN inhibits the early-stages of HIV-1 replication was evaluated using time-of-drug-addition assays and quantitative polymerase chain reaction (PCR) studies using acutely infected MT-2 cells.

2.2.1. Biophysical Characterization of LEN Binding to Recombinant HIV-1 Capsid Protein

The binding of LEN to recombinant HIV-1 CA protein and its effect on in vitro CA assembly were studied (m2.6.3, Section 2, PC-200-2022). Protein X-ray crystallography studies were performed using a covalently cross-linked CA hexamer protein that has been shown to closely approximate the structures of hexameric CA present within intact HIV-1 particles {Mattei 2016, Pornillos 2009}. Results indicated that LEN binds at the interface formed between two adjacent CA monomers within a CA hexamer. Lenacapavir forms extensive interactions with each of two adjacent CA monomers forming the interface. Surface plasmon resonance (SPR) experiments demonstrated that LEN binds with high affinity to cross-linked CA hexamer (K_D = 1.4 ± 0.6 nM). Consistent with an on-target interaction, the capsid M66I resistance mutation attenuated LEN binding to recombinant CA protein. Lenacapavir increased both the rate and extent of in vitro CA assembly, resulting in short, misshaped and heterogeneous polymers that differed from the uniformly long and well-organized CA tubes assembled in the absence of LEN. These findings indicate that LEN has the potential to interfere with HIV-1 replication by changing the kinetics of capsid assembly/disassembly, leading to an altered shape and/or stability of the conical capsid shell.

2.2.2. Inhibition of HIV-1 Early- and Late-Stage Replication Steps by LEN in MT-2 Cells

The in vitro antiviral activity of LEN was determined against the early stages, the late stages, and a complete single round of HIV-1 infection in the MT-2 T-cell line (m2.6.3, Section 2, PC-200-2021, Table 6). Lenacapavir showed potent antiviral activity against a single round infection using WT HIV-1 reporter virus but was inactive against the capsid M66I resistant HIV-1 variant (WT and mutant EC_{50} values of 0.025 nM and >50 nM, respectively). Lenacapavir retained full antiviral activity when the presence of the compound was restricted to the target cell infection phase, with an early-stage EC_{50} of 0.023 nM. When restricted to the late stage in virus producer cells, LEN showed a potent antiviral effect with a mean late stage EC_{50} of 0.439 nM. Thus, LEN targets both an early and late stage capsid-mediated event essential for HIV-1 replication. In contrast to the multi-stage antiviral activity observed with LEN, raltegravir (RAL) and ATV showed an antiviral effect specific only to the early or late stages of HIV-1 replication, but not both. Unlike RAL and nevirapine (NVP), LEN was also capable of irreversibly inactivating fully infectious cell-free HIV-1 virions with a mean virucidal EC_{50} of 25 nM (m2.6.3, Section 2, PC-200-2021). These data demonstrate that LEN mediates the potent suppression of both early and late stage essential processes in the HIV-1 replication cycle. This dual mechanism of action is unique among all clinically used antiretroviral classes and is detailed in the following two sections.

Table 6. Antiviral Activity of LEN at Distinct Stages of HIV-1 Replication Cycle in MT-2 Cells

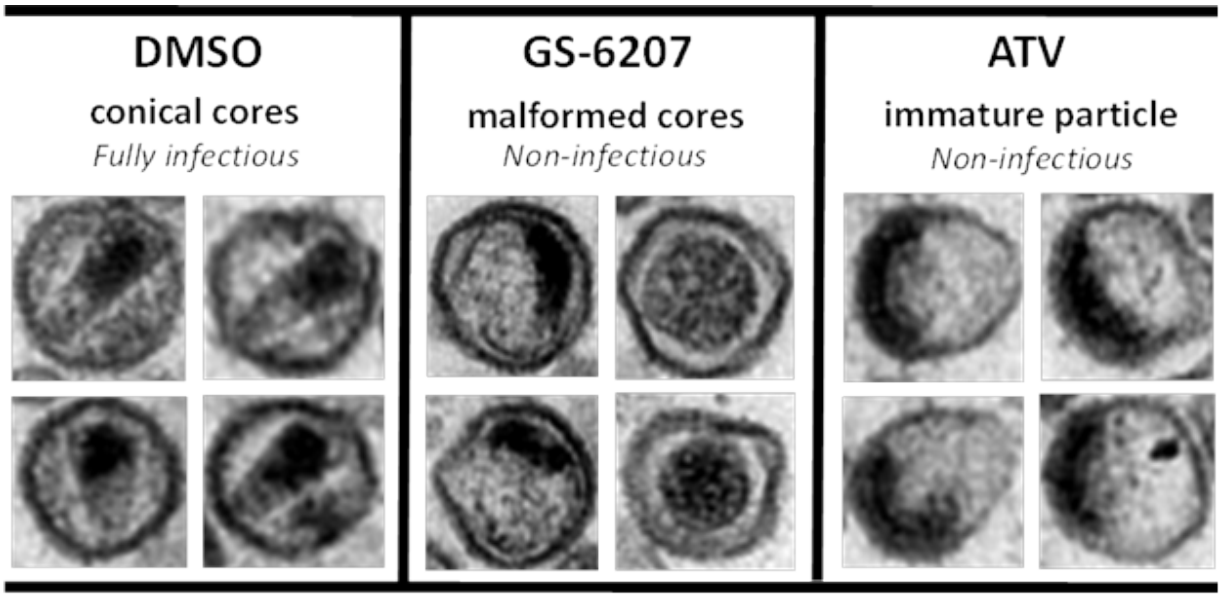
Compound[1]	EC_{50} (nM)[2]			
	Complete Replication Cycle (WT HIV-1)	Target Cell Infection (Early-Stage)	Virus Production and Maturation (Late-Stage)	Complete Replication Cycle (HIV-1 CA-M66I)
LEN	0.025 ± 0.003	0.023 ± 0.008	0.439 ± 0.181	> 50
RAL	1.7 ± 0.1	1.9 ± 0.8	> 1,000	1.8 ± 0.4
ATV	2.0 ± 1.0	> 1,000	2.3 ± 0.9	4.1 ± 0.8

1 ATV = atazanavir; RAL = raltegravir
2 EC_{50} and CC_{50} values represent mean (± SD) obtained from 3 independent experiments (Data from PC-200-2021).

2.2.3. Inhibition of Proper Capsid Core Formation and HIV-1 Assembly by LEN

The morphology of capsid structures was examined in ultrathin-sectioned HIV-1 (strain IIIB) produced in the presence and absence of LEN by transmission electron microscopy (m2.6.3, Section 2, PC-200-2023, Figure 2). The vehicle (dimethyl sulfoxide, [DMSO])-treated controls showed the expected conical capsids with an encased dark signal at the wide end of the cone consistent with negative staining of viral nucleic acids. In contrast, virions produced in the presence of LEN contained predominantly malformed capsid cores in which the nucleic acids were either regionally or diffusely confined within the irregularly shaped capsid core. This morphology was distinct from virus produced in the presence of an HIV-PI (ATV), which lacked any capsid-like structure. Virus produced in the presence of LEN or ATV was non-infectious when diluted below the EC_{50} concentration of each compound.

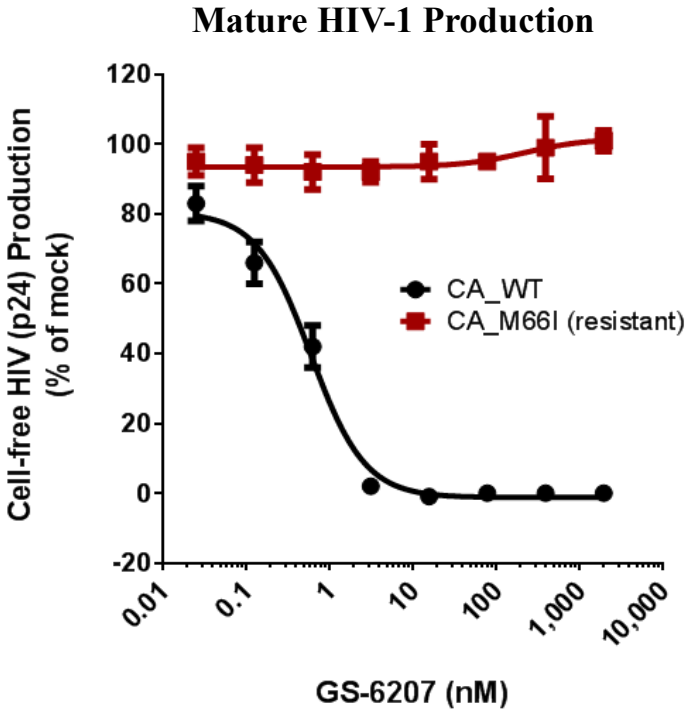
Figure 2. Representative Electron Micrographs Showing Capsid Morphology in HIV-1 Produced in the Presence and Absence of LEN



MT-2 cells chronically infected with HIV-1 IIIB strain (MT-2/IIIB) were incubated in the presence of 0.25% DMSO, 50 nM LEN (GS-6207) or 1 μM ATV for 4 days. Virus was pelleted by centrifugation, fixed, ultrathin sectioned, counter stained and examined on an electron microscope (Data from PC-200-2023).

The effect of LEN on virus production was assessed in HEK293T cells following transient transfection with plasmids encoding single-cycle HIV-1 reporter virus (m2.6.3, Section 2, PC-200-2023). Virus-producing HEK293T cells showed a dose-dependent reduction in the amount of mature HIV-1 released into the cell culture supernatant over a period of two days in the presence of LEN, with an EC_{50} of 0.305 nM as measured by p24 ELISA (Figure 3). Lenacapavir showed no cytotoxicity in HEK293T cells up to the highest concentration tested (10 μM) and did not inhibit the production of a drug-resistant HIV-1 variant with the capsid M66I mutation ($EC_{50} > 2$ μM), indicating that this late-stage effect is both virus-specific and requires the WT capsid domain. Western blot analyses of Gag cleavage products demonstrated that LEN did not inhibit Gag polyprotein cleavage in virus-producing cells. However, LEN did significantly reduce intracellular capsid (p24), and to a lesser extent, Gag (p55) levels by a mechanism that required LEN binding to the capsid domain of these viral proteins as these effects were abrogated with the capsid M66I resistant HIV-1 mutant.

Figure 3. Effect of LEN on the Production of Mature Extracellular WT and LEN-Resistant HIV-1 in HEK293T Cells[1]



1 Extracellular p24 levels represent the mean (± SD) obtained from 2 independent experiments (Data from m2.6.3, Section 1, PC-200-2023).

2.2.4. Effects of LEN on Reverse Transcription, Integration of Viral DNA and Accumulation of 2-LTR Circles

The mechanism(s) by which LEN inhibits the early stages of the HIV-1 replication cycle were investigated (m2.6.3, Section 2, PC-200-2024). Lenacapavir showed no effect on HIV-1 entry into PBMCs using a quantitative virus entry reporter assay, indicating that LEN targets a post-entry process essential for HIV-1 replication. Time-of-drug-addition assays demonstrated that LEN inhibits HIV-1 infection after reverse transcription but before provirus integration. Quantitative PCR studies using acutely infected MT-2 cells demonstrated that, unless using LEN concentrations in excess of 100-fold its EC_{50} value, LEN showed no effect on the accumulation of reverse transcription products, but profoundly reduced the accumulation of integration products and abortive 2-LTR circles, each of which is formed exclusively in the nucleus {Bukrinsky 1993} (Table 7). Thus, LEN primarily inhibits an essential process in the HIV replication cycle that occurs after reverse transcription, but before the integration of viral DNA. That LEN reduced the accumulation of 2-LTR circles rather than increasing these products of abortive integration, as observed with DTG, suggests that LEN inhibits the active transport of viral pre-integration complexes into the host cell nucleus. The nuclear translocation of intact capsids containing viral DNA is mediated in part by various host nuclear import factors (eg, CPSF6, NUP158, NUP358) that interact with HIV-1 capsid via the same binding site as that of the LEN molecule {Bhattacharya 2014, Matreyek 2013, Price 2012, Price 2014}

(m2.6.3, Section 2, PC-200-2022). Consequently, it is expected that LEN competes with these host cell factors for binding to HIV-1 capsid protein and thereby interferes with capsid-mediated nuclear import of pre-integration complexes, an essential step in the formation of an integrated provirus. Alternatively, this nuclear import block could originate, at least in part, from increased steric hindrance following LEN-mediated stabilization of CA oligomers within capsids docked at nuclear pore complexes.

Table 7. Effect of LEN vs. Control Inhibitors on the Accumulation of Products of Reverse Transcription, HIV Integration and Integration Failure

Compound[1]	Concentration Used (nM)[2]	Fold EC_{50}	Direct Effect on Reverse Transcription (RT)		Integration Failure	Direct Effect on Integration
			Early-RT (fold-change)[2]	Late-RT (fold-change)[2]	2-LTR Circles (fold-change)[2]	Alu-LTR (fold-change)[2]
DMSO	-	-	1.0	1.0	1.0	1.0
LEN	0.05	1.2	1.1	1.0	1.0	0.6
	0.25	6.1	1.0	0.8	0.4	0.06
	1.25	31	0.9	1.0	0.3	0.02
	2.5	61	0.6	0.7	0.3	0.01
	10	244	0.3	0.2	0.1	0.01
EFV	500	250	0.4	0.2	0.1	0.03
DTG	500	94	1.2	1.0	3.0	0.02
ATV	500	27	1.1	1.1	0.7	0.8

1 ATV = atazanavir; DMSO = dimethyl sulfoxide; DTG = dolutegravir; EFV = efavirenz
2 Copy numbers for HIV target amplicons were normalized against the corresponding copy numbers for the endogenous CCR5 gene from the same sample and expressed as a fold-change (FC) value relative to the DMSO-treated infected control (set to 100%) for each target amplicon measured. Values represent the mean of 3 replicates from a single experiment. Fold-change in target product accumulation is colored for FC ≥ 1.5, for FC > 0.1 to ≤ 0.5, and for FC ≤ 0.1. (Data from m2.6.3, Section 1, PC-200-2024).

2.3. LEN Cross-Resistance

2.3.1. Activity of LEN in HIV-1 Mutants Resistant to the 4 Main Drug Classes

The in vitro antiviral activity of LEN was determined in MT-2 cells against a broad spectrum of HIV-1 site-directed mutants (SDMs) and patient-derived HIV-1 isolates resistant to NRTIs, NNRTIs, INSTIs or PIs (m2.6.3, Section 2, PC-200-2027; n=18). Lenacapavir remained fully active against all 18 HIV-1 variants tested (Table 8), while representative control compounds from each of the four antiviral drug classes showed significant loss of antiviral activity when tested against viruses with mutations within their respective viral target protein. In addition, 40 HIV-1 clinical isolates with resistance against NRTIs, NNRTIs, INSTIs, and PIs (10 in each category; m2.6.3, Section 2, PC-200-2044) were tested using either the PhenoSense GT or PhenoSense Integrase single cycle assays (Monogram Biosciences). Here too, LEN retained full antiviral activity against the resistant isolates that was contrasted with the reduction in antiviral activity observed for control drugs from the classes tested (Table 9). These results demonstrate a non-overlapping resistance profile for LEN, indicating that LEN should retain full efficacy when challenged with HIV-1 mutants resistant to NRTIs, NNRTIs, INSTIs and/or PIs.

Table 8. Activity of LEN Against NRTI-, NNRTI-, INSTI-, and PI-Resistant HIV-1 Mutants

Antiretroviral Inhibitor Class	Drug-Resistant HIV-1 Mutant[1]	LEN	Positive Control	
		Fold-resistance[2]	Compound[3]	Fold-resistance[2]
NRTI	K65R	0.6	FTC	12.8
	M184V	0.5		>42
	6TAMs	0.4		4.1
NNRTI	K103N	0.3	EFV	14.4
	Y181C	1.7		3.3
	Y188L	0.5		>23
	L100I/K103N	0.5		>23
	K103N/Y181C	0.5		>23
INSTI	Y143R	0.5	RAL	10.2
	E138K/Q148K	0.8	EVG	>53
	G140S/Q148R	0.9		>53
	E92Q/N155H	0.9		>53
	N155H/Q148R	1.3		>53
	M50I/R263K	0.9		5.1
PI	I50V	0.6	DRV	31.8
	I84V/L90M	0.3	ATV	32.9
	I54V/V82S	0.4		32.4
	G48V/V82A/L90M	0.4		15.2

1 The antiviral activity of WT HIV-1 and HIV-1 encoding RT mutations (NRTI/NNRTIs), IN mutations (INSTIs), or PR mutations (PIs) was measured. 6TAMs: M41L, D67N, K70R, L210W, T215F, K219Q.

2 Mean fold-resistance values (mutant EC_{50}/WT EC_{50}) obtained from 3 independent experiments (Data from m2.6.3, Section 1, PC-200-2027).
> 2-fold, > 10-fold, > 50-fold

3 ATV = atazanavir; DRV = darunavir; EFV = efavirenz; EVG = elvitegravir; FTC = emtricitabine; RAL = raltegravir

Table 9. Relative Drug Susceptibility of LEN and Control ARVs in Isolates With Resistance to the Four Main ARV Classes

Drug Class	Drug	N	Median EC_{50} Fold Change from WT Control (Range)
CAI	LEN	40	0.62 (0.25 – 1.1)
NRTI	ABC	10	>6.8 (2.5 – >24)
	FTC	10	>37 (3.4 – >56)
	3TC	10	>40 (2.7 – >67)
	TFV	10	2.1 (0.75 – 18)
	ZDV	10	>19 (0.37 – >835)
NNRTI	EFV	10	>67 (3.5 – >67)
	ETR	10	>15 (3 – >178)
	NVP	10	>155 (38 – >155)
	RPV	10	>60 (3 – >60)
PI	ATV	10	>16 (2.2 – >170)
	DRV	10	30 (3 – 506)
	LPV	10	>83 (11 – >274)
	TPV	10	2.2 (0.7 – 20)
INSTI	BIC	10	1.6 (0.49 – 5.9)
	DTG	10	4.1 (0.71 – 14)
	EVG	10	>116 (25 – >116)
	RAL	10	>160 (20 – >200)

3TC: lamivudine; ABC: abacavir; ATV: atazanavir; BIC: bictegravir; CAI; capsid inhibitor; DRV: darunavir; DTG: dolutegravir; EFV: efavirenz; ETR: etravirine; EVG: elvitegravir; FTC: emtricitabine; INSTI: integrase strand transfer inhibitor; LEN: lenacapavir; LPV: lopinavir; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NVP: nevirapine; PI: protease inhibitor; RAL: raltegravir; RPV: rilpivirine; TFV: tenofovir; TPV: tipranavir; WT: wild-type (NL4-3); ZDV: zidovudine. (Data from PC-200-2044)

2.3.2. Activity of LEN in HIV-1 Clinical Isolates Containing Gag-Protease Sequences From ARV-Experienced and ARV-Naïve People with HIV

The antiviral potency of LEN was tested against a panel of treatment-experienced (TE) and treatment-naïve (TN) HIV-1 patient isolates that contained naturally occurring gag polymorphisms. Fifty-one patient-derived isolates comprising 36 TE and 15 TN HIV-1 sequences were cloned into pXXLAI, and their drug susceptibilities to LEN, protease inhibitors (DRV, ATV), and maturation inhibitors (BVM, GSK-3532795) were measured in a multicycle HIV-1 assay conducted in MT-2 cells.

In both TN and TE isolates, LEN exhibited similarly high potency (average EC_{50} of 0.088, and 0.089 nM, respectively) with minimal variability across all isolates (Table 10), that was very similar to the wild-type potency (EC_{50}: 0.095 nM). In contrast, the MIs BVM and GSK-3532795, showed significantly reduced potency in both TN and TE isolates, reflecting the occurrence of naturally existing gag polymorphisms associated with resistance to the MI class. Resistance to the PIs darunavir (DRV) and atazanavir (ATV) was high in TE isolates, as expected in the presence of PI resistance mutations, with mean fold-change EC_{50} above WT of 21 and 34, respectively, compared to fold-change of 1.0 for both drugs in TN isolates. Overall, these data indicate that the antiviral activity of LEN was not affected by the presence of naturally occurring pre-existing gag polymorphisms, or the presence of PI-resistance mutations, or the degree of treatment experience of the patients.

Table 10. Relative Drug Susceptibilities in Treatment-Experienced and Treatment-Naïve HIV-1 Isolates

	Mean Drug Susceptibilities (EC_{50} Fold-Change from Wild-Type, range)					
Isolate Type	**LEN (CAI)**	**DRV (PI)**	**ATV (PI)**	**BVM (MI)**	**GSK-795* (MI)**	**TAF (NRTI)**
TE (n=36)	0.9 0.4 – 1.8	21 0.5 – >112	35 0.5 – >66	40 0.5 – >64	94 0.5 – >333	0.8 0.3 – 1.4
TN (n=15)	0.9 0.6 – 1.6	1.0 0.5 – 1.8	1.0 0.5 – 1.9	42 1.7 – >64	72 1.1 – >333	0.9 0.5 – 1.5

CAI: capsid inhibitor; MI: maturation inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TE: treatment-experienced isolates; TN: treatment-naïve isolates. (Data from PC-200-2036)

2.3.3. Activity of LEN in HIV-1 Site-Directed Mutants and Clinical Isolates Containing Gag Cleavage Site Mutations

The antiviral potency of LEN was tested against a panel of site-directed mutant viruses (SDM; n=19) and HIV-1 clinical isolates from treatment-experienced people with HIV (PWH) (TE; n=24) containing gag cleavage site mutations (GCSMs) with or without protease inhibitor resistance (m2.6.3, Section 2, PC-200-2037). These SDMs included some combination of the Gag mutations L363F/M, A364V, Q430R, A431V, K436E/S, I437T/V, L449H/V/F, and P453L cloned into pXXLAI. Drug susceptibilities to LEN, protease inhibitors (DRV, ATV), and maturation inhibitors (BVM, GSK-3532795) were measured in a multicycle HIV-1 assay in MT-2 cells.

Lenacapavir displayed WT antiviral potency across this panel of 43 mutants with a mean fold-change in EC_{50} of 1.0 relative to the wild type virus (SDM: 1.1-fold, TE: 1.0-fold; Table 11). In contrast, the presence of GCSMs in some SDMs was associated with low-level resistance to PIs and low- to high-level resistance to MIs, while overall high-level resistance (mean fold-change >22) to both PIs and MIs was observed in TE isolates containing GCSMs. Overall, these data indicate that LEN susceptibility was not affected by the presence of GCSMs in the SDM and TE isolates tested.

Table 11. Relative Drug Susceptibilities in HIV-1 Site-Directed Mutants and Treatment-Experienced Isolates Harboring Gag Cleavage Site Mutations

Isolate Type	Mean Drug Susceptibilities (EC_{50} Fold-Change from Wild-Type, range)					
	LEN (CAI)	DRV (PI)	ATV (PI)	BVM (MI)	GSK-795* (MI)	TAF (NRTI)
SDM (n=19)	1.1 0.7 – 1.9	1.2 0.5 – 2.3	1.8 0.4 – 3.6	>12 0.3 – >64	31 0.7 – 267	0.9 0.5 – 1.4
TE With GCSM (n=24)	1.0 0.4 – 1.8	>22 0.5 – >112	>36 0.7 – >66	>40 0.5 – >64	>85 0.5 – >333	0.8 0.3 – 1.4

* GSK-795: GSK-3532795/BMS-955176
CAI: capsid inhibitor; GCSM: gag cleavage site mutations; MI: maturation inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; SDM: site-directed mutants; TE: treatment-experienced isolates. (Data from PC-200-2037)

2.4. LEN Resistance

2.4.1. In Vitro Selection for LEN Resistant HIV-1 Variants

In vitro dose escalation resistance selections with LEN, EFV, and EVG were performed in MT-2 cells infected with clonal HIV-1 strain HXB2D (m2.6.3, Section 2, PC-200-2025). The in vitro rates of viral resistance emergence were similar for all three compounds under conditions of low drug pressure but were considerably slower for LEN relative to EFV and EVG at higher drug concentrations (> 10-fold EC_{50}). At low LEN concentrations, the compound selected for virus encoding the capsid N74D variant, whereas at higher drug concentrations, LEN selected for virus encoding the capsid Q67H+N74D double mutant (Table 12). Phenotypic profiling of selected viral passages (P3-P10) demonstrated that the N74D and Q67H+N74D variants conferred an average of 5.3-fold and 119-fold resistance to LEN, respectively, with no change in susceptibility to any of the control inhibitors from other antiretroviral classes (EFV, EVG, and ATV) (Table 12).

Table 12. Genotypic and Phenotypic Profiles of Selected Viral Passages

Selected Virus	Duration of Selection (days)	Drug Conc. Reached, nM (Fold EC_{50})	Mutation(s) [1]	EC_{50} (nM) (Fold-Change Relative to WT)[2]			
				LEN	EFV	EVG	ATV
HXB2D	—	—	None	0.26 ± 0.15 (1.0)	1.67 ± 0.47 (1.0)	1.72 ± 0.41 (1.0)	6.40 ± 1.43 (1.0)
LEN P3	12	0.28 (4)	None	0.13 ± 0.1 (0.5)	1.33 ± 0.49 (0.8)	1.50 ± 0.57 (0.9)	6.70 ± 0.82 (1.1)
LEN P4	19	0.56 (8)	N74D	1.42 ± 0.71 (5.5)	0.63 ± 0.12 (0.4)	1.72 ± 1.53 (1.0)	4.30 ± 0.35 (0.7)
LEN P5	36	1.12 (16)	N74D	1.31 ± 0.54 (5.0)	0.63 ± 0.12 (0.4)	1.25 ± 0.21 (0.7)	3.17 ± 0.51 (0.5)

Selected Virus	Duration of Selection (days)	Drug Conc. Reached, nM (Fold EC_{50})	Mutation(s) [1]	EC_{50} (nM) (Fold-Change Relative to WT)[2]			
				LEN	EFV	EVG	ATV
LEN P6	54	2.24 (32)	N74D, T107N/T	1.79 ± 0.67 (6.9)	0.67 ± 0.06 (0.4)	0.93 ± 0.32 (0.5)	4.30 ± 0.17 (0.7)
LEN P7	80	4.48 (64)	Q67H, N74D	11.1 ± 5.6 (43)	0.52 ± 0.32 (0.3)	0.32 ± 0.05 (0.2)	3.10 ± 0.62 (0.5)
LEN P8	89	8.96 (128)	Q67H, N74D	19.8 ± 22.9 (76)	0.46 ± 0.23 (0.3)	0.28 ± 0.16 (0.2)	1.98 ± 0.95 (0.3)
LEN P9	96	17.9 (256)	Q67H, N74D	53.9 ± 28.6 (207)	0.58 ± 0.21 (0.4)	0.29 ± 0.06 (0.2)	2.34 ± 0.32 (0.4)
LEN P10	103	35.8 (512)	Q67H, N74D	39.3 ± 6.5 (151)	0.57 ± 0.17 (0.4)	0.32 ± 0.01 (0.2)	2.15 ± 0.23 (0.3)
EFV P12	81	1,843 (2,048)	L100I, K103N, T165I/T	0.24 ± 0.08 (0.9)	>500 (>299)	3.99 ± 0.46 (2.3)	3.90 ± 0.45 (0.6)
EVG P10	101	512 (512)	T66I, E92Q	0.15 ± 0.03 (0.6)	0.91 ± 0.12 (0.6)	186 ± 48 (108)	3.33 ± 0.25 (0.5)

EFV = efavirenz; EVG = elvitegravir; ATV = atazanavir
1 Relative to HIV-1 HXB2D sequence.
2 Fold-change values are calculated from the ratio of EC_{50} of the selected virus over the EC_{50} of HIV-1 HXB2D. The values represent the mean (± SD) obtained from 3 independent experiments (Data from PC-200-2025).

Resistance experiments were also performed at clinically relevant fixed drug concentrations in MT-2 cells and in human PBMCs (m2.6.3, Section 2, PC-200-2025). In MT-2 cells infected with clonal HIV-1 strain HXB2D, LEN was tested at concentrations corresponding to tissue culture equivalent IQs of 4, 8, 16, 24, and 40, with an IQ of 1 defined as a concentration corresponding to the EC_{95} for LEN (0.23 nM). The rate and frequency of viral breakthrough for fixed LEN concentrations was compared to that of four clinically approved antiretroviral inhibitors (emtricitabine [FTC], EFV, rilpivirine [RPV], and ATV), with each used at a fixed drug concentration equal to their respective tissue culture equivalent (ie, adjusted for protein binding) of the clinical plasma trough concentration (C_{min}) {Mulato 2016}. At a fixed concentration of 0.92 nM LEN (IQ of 4), a replicating virus emerged in 3 of 8 independent infected samples tested and each isolate encoded the capsid N74D mutation previously identified at low LEN concentrations in the dose escalation selections. No breakthrough virus emerged at any of the higher test concentrations of LEN. In comparison, parallel selections with FTC resulted in rapid emergence of the clinically relevant M184I/V reverse transcriptase (RT) variants in 7 of 8 infected samples, whereas a breakthrough of the Y181I RT variant was observed in the presence of RPV in 1 of 8 infected samples tested. Selections with fixed C_{min} concentrations of EFV, ATV and DTG each did not lead to any breakthrough of drug-resistant HIV-1 variants. These data suggest a high barrier to in vitro resistance emergence for LEN at concentrations above an IQ of 4.

Breakthrough studies were also performed at clinically relevant fixed drug concentrations in human PBMCs using six different patient-derived HIV-1 isolates and resulted in the emergence of a greater diversity of HIV-1 variants as compared to the experiments performed in MT-2 cells infected with a clonal HIV-1 isolate (Table 13). At an IQ of 4, LEN showed a breakthrough frequency lower than FTC and comparable to RPV. The frequency of viral breakthrough in the presence of LEN was substantially reduced (approximately 6-fold) in the presence of a two-fold higher drug concentration corresponding to an IQ of 8. These findings suggest that the C_{min} for LEN (corresponding to an IQ $\geq$ 6 or a 6x $paEC_{95} \geq 24.8$ nM) is expected to provide sufficient barrier to drug resistance development, comparable to other antiretrovirals presently used in the clinic.

Table 13. Viral Breakthrough Frequency at Fixed Concentrations of LEN in PBMCs Infected with Patient-derived HIV-1 Isolates

Drug[1]	Fixed Drug Exposure[2]	No. Breakthrough Samples From 36 Total Independent Selections Spanning 6 HIV-1 Isolates (Frequency)[2]	Mutation(s)[3] (No. of Breakthrough Samples with Each Genotype)
LEN	IQ = 4	17 (47%)	Q67H (10), N74D (4), L56I (1) Q67H+T107N (1) Q67H+N74S (1)
	IQ = 8	3 (8%)	N74D (3)
	IQ = 16	4 (11%)	N74D (2), M66I (1), K70N (1)
	IQ = 24	2 (6%)	L56 (1), M66I+Q67H (1)
FTC	C_{min}	29 (81%)	M184V (16), M184I (13)
RPV	C_{min}	12 (33%)	E138K (5), Y181C (2) E138K+M230I (2), L100I (1) E138K+V106A (1) Y181C+F227C (1)
EFV	C_{min}	3 (8%)	K103N (2), L100I+K103N (1)
DTG	C_{min}	0 (0%)	NA

1 FTC = emtricitabine; RPV = rilpivirine; EFV = efavirenz; DTG = dolutegravir
2 LEN test concentrations were 0.92, 1.9, 3.7, and 5.5 nM, corresponding to tissue culture equivalent fold EC_{95} values (IQs) of 4, 8, 16, and 24. Antiretroviral controls were tested at their respective tissue culture equivalent C_{min}: FTC (364 nM), RPV (8 nM), EFV (250 nM), DTG (73 nM). After 35 days in culture, samples were tested for breakthrough variants by assessing infectivity and genotype.
3 Relative to the sequence of each input patient-derived HIV-1 isolate (Data from m2.6.3, Section 1, PC-200-2025).

2.4.2. Activity of LEN Against LEN-Resistant Site-Directed HIV-1 Capsid Mutants in MT-2 Single Cycle Antiviral Assay

Lenacapavir was tested for antiviral activity against a panel of clonal site-directed HIV-1 variants encoding LEN resistance associated mutations identified in our in vitro selection experiments (Table 14). Relative to the WT virus, T107N and Q67H capsid variants conferred low level resistance to LEN (4- to 6.3-fold), K70N, N74D and the double mutant Q67H+N74S conferred moderate LEN resistance (22- to 32-fold), and L56I and M66I, as well as four additional double mutant viruses (M66I+Q67H, Q67H+N74D, Q67H+T107N, N74D+T107N),

all conferred high level LEN resistance (58- to >3,226-fold). Lenacapavir also showed 8.8-fold and 21-fold reduced activity relative to WT HIV-1, respectively, against the A105E and Q67Y variants identified during in vitro drug selection with structurally similar analogues of LEN but not with LEN itself. The control antiretroviral EFV remained fully active against all of the capsid HIV-1 variants tested, with mean fold-resistance values ranging from 0.6 to 1.9. All tested LEN-resistant HIV-1 variants except Q67H showed significantly reduced infectivity in MT-2 cells (4-50% of WT virus), suggesting that the majority of the identified LEN resistance associated mutations severely compromise virus fitness.

Table 14. Single-Cycle Infectivity of LEN Resistant Mutants of HIV-1 and Susceptibility to LEN in MT-2 Cells

HIV-1 Capsid Genotype	Single-Cycle Infectivity (% of WT)[1]	LEN	
		EC_{50} (nM)[1]	Fold-resistance[2]
WT	100	0.031 ± 0.003	-
L56I	8 ± 1	7.40 ± 0.67	239
M66I	4 ± 2	> 100	> 3,226
Q67H	121 ± 16	0.196 ± 0.028	6.3
Q67Y	39 ± 5	0.635 ± 0.040	21
K70N	7 ± 1	0.741 ± 0.214	24
N74D	50 ± 11	0.682 ± 0.106	22
A105E	9 ± 3	0.272 ± 0.044	8.8
T107N	44 ± 4	0.124 ± 0.006	4.0
M66I + Q67H	8 ± 2	> 100	> 3,226
Q67H + N74D	29 ± 6	34.1 ± 2.6	1,099
Q67H + N74S	33 ± 5	0.996 ± 0.176	32
Q67H + T107N	41 ± 7	1.91 ± 0.12	62

1 Infectivity and EC_{50} values represent the mean (± SD) obtained from 3 independent experiments.
2 Mean fold-resistance values (mutant EC_{50}/WT EC_{50}) obtained from 3 independent experiments (Data from PC-200-2026).
> 2-fold, > 10-fold, > 50-fold, > 100-fold, > 1000-fold

2.4.3. Activity of LEN Against LEN-Resistant Site-Directed HIV-1 Capsid Mutants in PhenoSense Gag-Pro Single Cycle Antiviral Assay

Susceptibility to LEN of in vitro selected LEN-resistant SDMs (n=12) was tested in the single cycle antiviral assay from Monogram Biosciences (m2.6.3, Section 2, PC-200-2046). Replication capacity and LEN susceptibility data of the SDMs in the PhenoSense assay displayed similar trends compared to the data obtained in the MT-2 single cycle assay (Table 14). The susceptibility of these 12 SDMs to LEN ranged from 3.8- to >2757-fold relative to the WT, whereas each SDM remained fully susceptible to the PIs ATV and DRV (Table 15). These data indicate a lack of cross-resistance of LEN-resistant mutants to the protease inhibitor class.

Table 15. Replication Capacity and Drug Susceptibilities of LEN-Resistance Associated Mutations in the PhenoSense Assay

HIV-1 Capsid Genotype	Replication Capacity (% of WT)	Relative Drug Susceptibilities (fold change from WT control)		
		LEN	ATV	DRV
L56I	3.6%	204	1.5	1.6
M66I	1.5%	>2757	0.9	1.3
Q67H	58.0%	4.8	1.2	0.9
Q67Y	10.0%	24	1.4	1.2
K70N	1.2%	N/A		
N74D	N/A	16	0.9	1.1
A105E	32.0%	4.0	1.1	1.7
T107N	32.0%	3.8	1.2	1.5
M66I+Q67H	N/A	1594	1.2	1.2
Q67H+N74D	N/A	>2757	1.0	1.1
Q67H+N74S	15.0%	20	1.1	1.5
Q67H+T107N	N/A	87	1.2	1.2
Median Fold Change		24	1.2	1.2

ATV: atazanavir; DRV: darunavir; LEN: lenacapavir; N/A: not available; WT: wild-type. (Data from PC-200-2046)

2.4.4. Activity of LEN Against LEN-Resistant Site-Directed HIV-1 Capsid Mutants in MT-2 Multicycle Antiviral Assay

The antiviral potency of LEN, PIs, and MIs was tested in a multicycle (MC) HIV-1 antiviral assay in MT-2 cells against a panel of replication-competent site-directed mutant viruses (SDM; n = 12) containing resistance mutations to LEN or a structurally similar analogue of LEN (m2.6.3, Section 2, PC-200-2045). Close to half of these SDMs (5 of 12; L56I, M66I, K70N, A105E, and M66I+Q67H) did not show adequate infectivity in this MC antiviral assay and thus their drug susceptibility could not be evaluated. These 5 mutants also displayed reduced replication capacity in single-cycle (SC) antiviral assays conducted separately (m2.6.3, Section 2, PC-200-2026; Table 14). For the remaining 7 mutant viruses, relative susceptibility to LEN ranged from 4.1- to 306-fold change (mean: 52.4-fold) in these mutants compared to the wild type in the MC assay (Table 16), which correlated well with LEN phenotypic data obtained for these mutants in the SC assay. Importantly, no cross-resistance with protease inhibitors or maturation inhibitors was noted with these LEN-resistant mutants indicating that the replication of LEN-resistant mutants can be effectively suppressed by other classes of drugs.

Table 16. Relative Drug Susceptibilities of LEN-Resistant Site-Directed HIV-1 Capsid Mutants in MT-2 Multicycle Antiviral Assay

Average EC_{50} Fold Change Compared to WT Control[a]									
		CAI	PI				MI		NRTI Control
Mutant ID	CA Mutations	LEN	DRV	ATV	NFV	SQV	BVM	GSK-795[b]	TAF
114	Q67H	5.7	1.4	1.7	1.2	1.3	1.1	1.2	1.5
115	Q67Y	10.3	1.1	1.4	0.9	1.6	0.7	0.9	1.5
117	N74D	14.3	1.1	0.8	0.4	0.8	0.7	0.9	1.6
120	T107N	4.1	1.1	1.0	0.7	0.8	1.3	1.1	0.9
122	Q67H+N74D	306	1.1	0.9	0.9	1.3	0.6	1.1	1.2
123	Q67H+N74S	8.4	2.1	1.9	1.9	1.7	1.0	1.6	1.3
124	Q67H+T107N	18.2	1.2	0.8	0.7	0.9	0.5	0.8	0.7
Mean Fold Change		52.4	1.3	1.2	1.0	1.2	0.8	1.1	1.2

a Average of at least 3 independent experiments
b GSK-795: GSK-3532795/BMS-955176
CA: capsid; CAI: capsid inhibitor; MI: maturation inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; WT: wild-type. (Data from PC-200-2045)

2.4.5. Replication Capacity of WT and LEN Resistant HIV-1 Mutants in Primary Target Cells

The in vitro replication capacity of WT HIV-1 and LEN resistance associated HIV-1 variants was investigated in primary human $CD4^+$ T cells and in monocyte-derived macrophages (m2.6.3, Section 2, PC-200-2026). Except for the Q67H variant that confers low level resistance to LEN, the six other capsid mutants tested (ie, L56I, M66I, N74D, Q67H+N74D, Q67H+T107N, N74D+T107N) displayed substantially diminished replication capacity in both $CD4^+$ T-lymphocytes (0.03-28% of WT virus) and macrophages (1.9-72% of WT virus) for the duration of the experiment (19 days). Importantly, the replicative fitness of these mutants was not significantly restored in the presence of LEN in either cell type. Replication capacity (RC) data available from 3 mutants (L56I, M66I, and Q67H) in both MC and SC assays (Sections 2.4.3 and 2.4.5) indicated the same rank order, with M66I RC of 1.5% and 0.4%, L56I RC of 3.6% and 3.0%, and Q67H RC of 58% and 100%, respectively. These data indicate that the in vitro replication capacity of the majority of the capsid HIV-1 mutants tested, including those conferring moderate to high-level LEN resistance, is severely compromised relative to the WT virus in two primary human target cell types of HIV-1 infection.

2.4.6. Bioinformatics Assessment of HIV-1 Capsid Protein Conservation and Polymorphisms in the LEN Binding Site Across HIV-1 Subtypes

A publicly available source of 10,512 HIV-1 capsid population sequences representing 8 major subtypes (A1, B, C, D, F1, G, CRF01_AE, and CRF02_AG) was used to evaluate the conservation of the CA protein and the LEN binding site (m2.6.3, Section 2, PC-200-2032). The overall sequence identity of CA protein across these 8 subtypes was found to be high (≥91%). Of the 24 amino acid residues in the LEN binding site, 20 residues (83%) had a sequence conservation between 94% and 100% across all 8 subtypes, including all 7 capsid amino acid positions associated with in vitro resistance to LEN (ie, L56 [100%], M66 [100%], Q67 [99% for CRF01_AE, 100% for other subtypes], K70 [100%], N74 [100%], A105 [100%], and T107 [94-99%]). None of the T107 substitutions identified was the T107N variant that is associated with 3.8- to 4.1-fold reduced susceptibility to LEN. The remaining 4 binding site residues (S41, Q50, T54, and Y169) were mutated in >5% of patients in at least one major subtype. SDMs with the identified substitutions were created (S41A, S41I, S41L, S41T, S41V, Q50A, Q50E, Q50G, Q50H, Q50S, Q50T, Q50Y, T54L, T54M, T54S, T54V, N57H, T107A, T107S, T107V, Y169F, L172V, Q179T, K182R) and their impact on the susceptibility to LEN was tested in the MT-4 single cycle assay (m2.6.3, Section 2, PC-200-2038). Lenacapavir susceptibility in these SDMs was within the WT range (0.7- to 2.4-fold change) for all mutants except for Q50E (3-fold from WT) and N57H (>5000-fold from WT). Notably, these two LEN resistant variants each showed reduced infectivity in MT-4 cells (20% to 30% of WT) and were detected in HIV-1 at only a low (1%) prevalence exclusively in either subtype C (Q50E) or subtype D (N57H) isolates.

Deep sequence analysis of capsid from 104 patient isolates of mostly HIV-1 subtype B showed essentially similar results as the population sequence analysis. In addition, the only LEN resistance associated mutation detected by deep sequencing was the capsid T107N substitution detected in a single patient. These data indicate a high degree of sequence conservation within the LEN binding site in HIV-1 capsid, indicating a very low frequency of potentially pre-existing resistance to LEN among HIV-1 clinical isolates.

3. SECONDARY PHARMACODYNAMICS

3.1. Antiviral Activity and Selectivity of LEN Against Non-HIV Viruses

To assess whether LEN has any activity against viruses other than HIV, LEN was tested against hepatitis B and C viruses, human rhinovirus serotype 16 (HRV-16), and respiratory syncytial virus strain A2 (RSV A2) in cell-based assays (m2.6.3, Section 3, PC-200-2029). Lenacapavir did not display selective in vitro antiviral activity against HCV or HRV up to the highest concentration tested (EC_{50} > 29 – 50 µM). Lenacapavir demonstrated low micromolar antiviral activity against HBV and RSV, with EC_{50} values and corresponding CC_{50}/EC_{50} ratios of 1.5 µM and >34, and 8.2 µM and >6.1, respectively (Table 17). These data indicate that LEN is approximately 29,000-fold more active against HIV-1 compared to HBV and therefore should not exert any clinical activity against HBV at drug exposures relevant for the inhibition of HIV.

Table 17. Antiviral Activity and Selectivity of LEN Against Non-HIV-1 Viruses

Virus	Cell Line	Positive Control[1]		LEN[1]		
		Compound	EC_{50} (nM)	EC_{50} (nM)	CC_{50} (µM)	Selectivity[2]
HCV 1b replicon	Huh7: Con1/ SG-hRLucNeo	ITMN-191	1.0 ± 0.5	> 29,000	> 44.4	>580,000
HCV 2a replicon	Huh7: JFH-1/ hRLucNeo2a	ITMN-191	21 ± 3	> 31,600	> 44.4	>632,000
HBV	HepG2-NTCP	Lamivudine	35 ± 19	1,462 ± 256	> 50	29,240
HRV-16	H1-HeLa	Rupintrivir	68 ± 22	> 50,000	> 50	>1,000,000
RSV A2	HEp-2	YM-53403	250 ± 24	8,199 ± 3,313	> 50	164,000

1 EC_{50} and CC_{50} values represent the mean (± SD) obtained from 3 independent experiments (Data from PC-200-2029).
2 Selectivity is calculated as EC_{50} against other virus divided by mean EC_{50} against 23 HIV-1 isolates in PBMCs (0.05 nM, data presented in Table 3).

3.2. In Vitro Cytotoxicity

The in vitro cytotoxicity of LEN was evaluated in multiple human cell types, including the MT-4 T-lymphoblastoid cell line, primary $CD4^+$ T-lymphocytes and monocyte-derived macrophages, and in non-target cell lines and primary hepatocytes.

3.2.1. Cytotoxicity and Selectivity of LEN in MT-4 T-Cells

The in vitro cytotoxicity of LEN was assessed in a 5-day cell viability assay in uninfected MT-4 cells (m2.6.3, Section 2, PC-200-2018). The concentration that resulted in 50% cell death (CC_{50} value) was 26.6 µM for LEN in MT-4 cells. When the anti-HIV-1 activity of LEN is taken into account, these data indicate that LEN has a selectivity index (CC_{50}/EC_{50}) of 140,000 in MT-4 cells (Table 18). The selectivity values for three clinically approved anti-HIV agents (EFV, DTG and ATV) assayed in parallel were 9.4- to 30-fold lower than those observed for LEN.

Table 18. Cytotoxicity and Selectivity of LEN in the MT-4 T-Cell Line

Compound[1]	MT-4 T-Cell Line	
	CC_{50} (µM)[2]	Selectivity[3]
LEN	26.6 ± 14.2	140,000
EFV	20.6 ± 4.6	14,930
DTG	15.3 ± 5.0	7,970
ATV	50.5 ± 8.1	4,720

1 EFV = efavirenz; DTG = dolutegravir; ATV = atazanavir
2 CC_{50} values represent the mean (± SD) obtained from at least 19 independent experiments (Data from m2.6.3, Section 1, PC-200-2018).
3 CC_{50}/EC_{50} ratio. Corresponding standard resolution EC_{50} values are presented in Table 1.

3.2.2. Cytotoxicity and Selectivity of LEN in Primary Human CD4+ T-Lymphocytes and Monocyte-Derived Macrophages

To assess the cytotoxicity of LEN in the natural target cells for HIV-1 infection, cytotoxicity assays were performed in uninfected primary human CD4+ T-lymphocytes and monocyte-derived macrophages following 7-day and 12-day incubations, respectively (m2.6.3, Section 2, PC-200-2019). Lenacapavir displayed a CC_{50} value of >50 µM in each of these primary target cells, resulting in a selectivity index (CC_{50}/EC_{50}) of >833,330 in $CD4^+$ T-lymphocytes and >1,670,000 in macrophages (Table 19). In these primary target cells, LEN was at least 22-fold more selective than three clinically approved anti-HIV agents (EFV, DTG, and ATV) assayed in parallel.

Table 19. Cytotoxicity and Selectivity of LEN in Primary Human Target Cells

Compound[1]	$CD4^+$ T-Lymphocytes		Monocyte-Derived Macrophages	
	CC_{50} (µM)[2]	Selectivity[3]	CC_{50} (µM)[2]	Selectivity[3]
LEN	> 50	> 833,330	> 50	> 1,670,000
EFV	17.4 ± 6.8	14,150	22.2 ± 14.1	76,200
DTG	15.8 ± 3.9	16,460	34.7 ± 21.6	18,560
ATV	29.8 ± 16.7	4,330	36.6 ± 23.1	4,400

1 EFV = efavirenz; DTG = dolutegravir; ATV = atazanavir
2 CC_{50} values represent the mean (± SD) obtained from 3 independent donors (Data from PC-200-2019).
3 CC_{50}/EC_{50} ratio. Corresponding EC_{50} values are presented in Table 2.

3.2.3. Cytotoxicity and Selectivity of LEN in Human PBMCs

The cytotoxicity of LEN was also measured in human PBMCs in the resting state and upon mitogen activation (m2.6.3, Section 2, PC-200-2019). The cytotoxicity of LEN in unstimulated and stimulated PBMCs from three independent donors was similar to that observed in primary $CD4^+$ T-lymphocytes and macrophages, with CC_{50} values of >44 µM and >50 µM, respectively (Table 20). When the mean anti-HIV-1 activity of LEN is taken into account, these data indicate that LEN has a selectivity index (CC_{50}/EC_{50}) of at least >888,000 in human PBMCs.

Table 20. Cytotoxicity and Selectivity of LEN in Human PBMCs

Compound	Resting PBMCs		Activated PBMCs	
	CC_{50} (µM)[1]	Selectivity[2]	CC_{50} (µM)[1]	Selectivity[2]
LEN	> 44.4	> 888,000	> 50	> 1,000,000
Puromycin	0.6 ± 1.5	-	0.2 ± 0.1	-

1 CC_{50} values represent the mean (± SD) obtained from 3 independent donors (Data from m2.6.3, Section 2, PC-200-2019).
2 CC_{50}/EC_{50} ratio. Corresponding mean EC_{50} value against 23 HIV-1 isolates in PBMCs (0.05 nM) is presented in Table 3.

3.2.4. Cytotoxicity and Selectivity of LEN in Non-Target Human Cell Lines and Primary Cells

The cytotoxicity of LEN was assessed in four non-target human cell lines, including two hepatoma cell lines (Huh7 and Gal-HepG2), a prostate cancer cell line (Gal-PC-3), a normal embryonic lung fibroblast line (MRC-5), and in primary human hepatocytes (m2.6.3, Section 3, PC-200-2028). Notably, two of these human cell lines (Gal-HepG2 and Gal-PC-3) were adapted to grow in glucose-free galactose-containing medium in order to increase the sensitivity of these cells to inhibitors of mitochondrial oxidative phosphorylation {Marroquin 2007, Nakabayashi 1982}. Lenacapavir did not display any significant cytotoxicity in the four tested cell lines or in primary human hepatocytes from three independent donors, with CC_{50} values in each cell line > 44 µM and >50 µM in primary human hepatocytes (Table 21). When the mean anti-HIV-1 activity of LEN in human PBMCs is taken into account (Table 3), these data indicate that LEN has a selectivity index (CC_{50}/EC_{50}) of >730,000 in each of the non-target human cell lines and primary human hepatocytes tested.

Table 21. Cytotoxicity of LEN in Multiple Human Cell Lines and Primary Cells Non-permissive to HIV-1 Infection

Compound	CC_{50} (µM)[1]				
	Huh7 Hepatoma Cell Line	Gal-HepG2[2] Hepatoma Cell Line	Gal-PC-3[2] Prostate Cancer Cell Line	MRC-5 Lung Fibroblast Cell Line	Primary Hepatocytes
LEN	> 44.4	> 44.4	> 44.4	> 44.4	> 50
Puromycin	0.5 ± 1.7	1.0 ± 1.7	0.4 ± 1.5	0.3 ± 1.5	1.6 ± 0.6

1 CC_{50} values represent the mean (± SD) obtained from 3 independent experiments (Data from PC-200-2028).
2 Galactose-adapted cell lines for enhanced sensitivity to mitochondrial toxicity
3 Puromycin was used as a positive control for cytotoxicity.

3.3. In Vitro Receptor Binding Potencies

A Lead Profiling Safety Screen was conducted to evaluate the activity of LEN against a panel of 87 targets (m2.6.3, Section 3, PC-200-2012). A single concentration of 10 µM was assessed to evaluate significant responses (>50% inhibition or induction). There were no significant responses for any of the targets evaluated. With an observed human therapeutic C_{max} of 136 ng/mL (0.140 µM) (free C_{max} of 1.98 ng/mL [0.002 µM]), after administration of the oral loading dose and a 927 mg subcutaneous (SC) dose, there is > 4,000-fold margin, and thus no clinically significant target inhibition or induction is likely.

4. SAFETY PHARMACOLOGY

To support the proposed clinical development plan, the nonclinical safety profile of LEN was characterized in studies evaluating the potential pharmacologic effects on specific organ systems. Study designs and parameters evaluated were consistent with accepted principles and practices as outlined in the International Conference on Harmonization (ICH), and United States Food and Drug Administration Center for Drug Evaluation and Research (US FDA CDER). All studies were conducted in accordance with US FDA Good Laboratory Practice (GLP) regulations.

Rat and dog were selected for the in vivo investigations based on evaluation of in vitro and in vivo pharmacokinetic (PK) properties and the exposures achievable in each species. As no gender-specific PK differences have been observed in rats, use of male rats only in the CNS and respiratory safety pharmacology studies was acceptable. Studies were conducted by the SC route of administration. The periods of assessments were later than those selected for immediate release drug products to ensure evaluation during the rise in LEN plasma concentrations and/or steady state. Margins were calculated using the C_{max} free fraction (1.98 ng/mL) achieved after the 6-month clinical oral loading and subcutaneous dosing regimen (CTRA-2021-1054 LEN PopPK).

4.1. Central Nervous System

As part of the study "Four-Dose (Twice Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of LEN in Wistar Han Rats" (m2.6.7, Section 7, TX-200-2005), a functional observation battery was conducted, and locomotor activity was assessed. Male rats (10/group) were subcutaneously administered 0 (vehicle, 2% [w/v] poloxamer 188 prepared in 0.9% [w/v] sodium chloride, in sterile water for injection), 10, 30 and 100 mg/kg/dose every other week for 4 injections. On each day of dosing, the pH of the LEN dose formulations was adjusted with 400 mM phosphate buffer. Rats were assessed once during the predose phase, once approximately 72 hours after dosing on days 1 and 43, and once within the last 4 days of study week 12. The maximum time to reach C_{max} (T_{max}) ranged from 48 to 168 hours.

No LEN-related alterations in quantitative evaluations occurred. No LEN -related qualitative elicited behaviors, handheld observations, home cage observations, or open field observations were noted. No LEN -related alterations occurred on locomotor activity.

The NOEL for CNS changes is 100 mg/kg for repeat SC administration. Exposures (based on C_{max} free fraction) at the no-observed-effect levels (NOEL) for CNS endpoints was 1.4-fold higher than the free C_{max} after the 6-month clinical oral loading and subcutaneous dosing regimen.

4.2. Cardiovascular System

4.2.1. In Vitro

The in vitro effects of compounds on the hERG (human ether-à-go-go-related gene) channel current (a surrogate for I_{Kr}, the rapidly activating delayed rectifier cardiac potassium current) are typically assessed using DMSO solutions of test article at near-physiological temperature. The patch-clamp technique is used to measure the peak hERG tail current amplitude prior to and following exposure to vehicle [0.3% DMSO v/v in HEPES-buffered Physiological Salt Solution; HB-PS] or test article. Lenacapavir DMSO/buffer formulations ≥ 0.1 µM were suspensions and not solutions and thus a GLP hERG assay was not conducted with LEN. The in vivo dog cardiovascular study is sufficient to assess potential cardiovascular toxicity due to LEN.

4.2.2. In Vivo

As part of the study "Four-Dose (Twice Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of LEN in Beagle Dogs" (m2.6.7, Section 7, TX-200-2006), effects on the cardiovascular system using safety pharmacology endpoints were evaluated. Male and female dogs (6/group) were subcutaneously administered 0 (vehicle, 2% [w/v] poloxamer 188 prepared in 0.9% [w/v] sodium chloride, in sterile water for injection), 10, 30 and 100 mg/kg/dose of LEN every other week for 4 injections. All high-dose males and 2 of 6 high-dose females (the only females dosed) underwent an early sacrifice because 100 mg/kg/dose exceeded the maximum-tolerated dose volume. The remaining 4 high-dose females were not dosed and were returned to the stock colony (see 2.6.6, Section 3.3). Electrocardiograms (ECGs) using jacketed external telemetry (JET) procedures were collected once during the predose phase and on Study Days 1, 15, 29, 43, and 82 on conscious animals. Blood pressure was determined via tail cuff once during the predose phase, once on day 43 at approximately 7 hours post dose, and once during study week 12. At the predose phase and Study Day 82 JET ECG intervals, data were recorded for at least 29 hours starting by 07:30. On Study Days 1, 15, 29, and 43, JET ECG recordings started at least 1.5 hours prior to room entry for dosing and were collected through at least 27 hours postdose.

For qualitative evaluation of ECG data, 1 to 1.5 minutes of continuous ECG data for each animal were isolated as follows:

- Predose Phase: Prior to dosing and approximately 2, 4, 6, 12, 16, and 21 hours postdose (based on the nominal dose time).
- Study Day 1: Prior to dosing and approximately 2, 4, 6, 12, 16, and 21 hours postdose (all animals in Groups 1, 2, and 3, and all dosed animals [6 males and 2 females] in Group 4).
- Study Days 15, 29, and 43: Prior to dosing and approximately 12, 16, and 21 hours postdose (all animals in Groups 1, 2, and 3).
- Study Day 82: Prior to dosing and approximately 2, 4, 6, 12, 16, and 21 hours postdose (based on the nominal dose time).

No ECG or blood pressure changes were attributed to LEN. The NOEL for ECG and blood pressure changes is 100 mg/kg for a single SC administration and 30 mg/kg for repeat SC administration.

Based on the LEN C_{max} in male and female dogs after the first 100 mg/kg SC dose (4,700 ng/mL), exposure (based on free fraction) at the NOEL was approximately 20-fold higher than the free C_{max} after the 6-month clinical oral loading and subcutaneous dosing regimen.

4.3. Respiratory System

Wistar Han rats (8 males/group) were administered a dose via SC injection of 0 (vehicle, 2% [w/v] poloxamer 188 prepared in 0.9% [w/v] sodium chloride, in sterile water for injection), 10, 30 or 100 mg/kg LEN (m2.6.3, Section 4.2, PC-200-2014). Assessment of respiratory function, measured using head-out plethysmography, was based on analysis of tidal volume (mL), respiration rate (breaths/minute), and minute volume (mL/minute). Plethysmography data were collected continuously for at least 2 hours on the day prior to dosing (baseline), consecutively from approximately 22.5 to 28.5 hours postdose, and for at least 2 hours beginning approximately 70.5 hours postdose. Parameters were analyzed as 30-minute averages at baseline, 22.5 to 28.5 hours and 70.5 to 72 hours postdose.

Administration of 100 mg/kg LEN to male Crl:WI(Han) rats via a single SC injection was associated with a minor non-statistically significant increase in tidal volume and a decrease in respiration rate which persisted through the assessed 23.5 through 28.5 and 72 hour postdose time period. No LEN -related changes in tidal volume or respiration rate were noted in animals administered ≤ 30 mg/kg up to 72 hours postdose. There was no LEN -related changes in minute volume in animals administered ≤ 100 mg/kg. Based on these results and using TK data from the 6-week rat toxicity study, the NOEL for respiratory effects is 30 mg/kg LEN in male rats; the NOAEL is 100 mg/kg. The free C_{max} at 30 and 100 mg/kg is 0.57 and 1.4, respectively, higher than the free C_{max} after the 6-month clinical oral loading and subcutaneous dosing regimen.

5. PHARMACODYNAMIC DRUG INTERACTIONS

5.1. Combination Study of LEN with HIV Antiviral Drugs

The in vitro anti-HIV-1 activity of LEN was examined in combination with clinically approved agents from each of the four major classes of antiretrovirals (NRTIs, NNRTIs, INSTIs, and PIs) (m2.6.3, Section 5, PC-200-2030, Table 22). Pair-wise combination of LEN with an NRTI (TAF) showed moderate synergistic anti-HIV-1 activity when tested in vitro using MT-2 cells infected with HIV-1 (IIIB strain). Lenacapavir exhibited highly synergistic activity when combined with an NNRTI (EFV), an INSTI (DTG), or a PI (DRV). Importantly, no antagonistic antiviral interaction was found between LEN and these four clinically relevant classes of antiretrovirals. These results support the clinical investigation of LEN in combination with other antiretroviral agents.

Table 22. In Vitro Anti-HIV-1 Activity of LEN in Combination with Selected Antiretrovirals

Drug Combination[1]	Synergy/Antagonism Volume ($\mu M^2.\%$)[2]		Combination Effect
	Type	Mean ± SD	
LEN + TAF	Synergy Antagonism	87 ± 32 -8 ± 7	Moderately Synergistic
LEN + EFV	Synergy Antagonism	101 ± 40 -8 ± 8	Highly Synergistic
LEN + DTG	Synergy Antagonism	116 ± 13 -8 ± 7	Highly Synergistic
LEN + DRV	Synergy Antagonism	119 ± 39 -3 ± 3	Highly Synergistic
Control Combinations[1]			
LEN + LEN	Synergy Antagonism	18 ± 8 -16 ± 6	Additive
TAF + EVG	Synergy Antagonism	164 ± 24 -3 ± 3	Highly Synergistic
RBV + D4T	Synergy Antagonism	0 ± 0 -398 ± 23	Highly Antagonistic

1 TAF = tenofovir alafenamide; EFV = efavirenz; DTG = dolutegravir; DRV = darunavir; EVG = elvitegravir; RBV = ribavirin; D4T = stavudine
2 The synergy/antagonism volumes represent the mean (± SD) of 3 independent experiments (Data from PC-200-2030).

6. CONCLUSIONS

Lenacapavir is a novel potent and selective inhibitor of HIV-1 capsid function, a mechanism that is orthogonal and complementary to existing anti-HIV therapies. The compound binds with high affinity to recombinant HIV-1 CA protein and increases both the rate and extent of in vitro CA assembly, resulting in the formation of poorly organized CA polymers relative to assembly products formed in the absence of LEN. Other key characteristics of LEN include:

- Absence of cytotoxicity in T cell line (MT-4) and in unstimulated and stimulated human PBMCs, with respective CC_{50} values of >26 μM, >44 μM and >50 μM. No cytotoxicity in several non-target human cell lines of different tissue origin

- Potent and selective antiviral activity in multiple cell types with EC_{50} ranging from 0.030 to 0.190 nM and selectivity (CC_{50}/EC_{50}) ranging from 140,000 to >1,670,000

- Potent antiviral activity at both early and late stages of HIV-1 replication, with EC_{50} values of 0.023 nM and 0.439 nM, respectively

- Potent antiviral activity against HIV-1 clinical isolates representing major HIV-1 subtypes (PBMCs: mean EC_{50} value of 0.05 nM, ranging from 0.02 to 0.16 nM; HEK293T cells: mean EC_{50} value of 0.238 nM, ranging from 0.148 to 0.357 nM)

- Antiviral activity unaffected by the presence of resistance mutations to other drugs classes (NRTIs, NNRTIs, INSTIs, PIs, or MIs)

- Antiviral activity unaffected by the presence of naturally-occurring Gag polymorphisms in contrast to compounds from the MI class, confirming that LEN and MIs exert their antiviral activity through different mechanisms

- Antiviral activity unaffected by the presence of Gag cleavage site mutations associated with PI treatment or MI resistance

- In vitro resistance selections with LEN identified 7 mutations in CA: L56I, M66I, Q67H, K70N, N74D/S, and T107N singly or in dual combination, associated with reduced susceptibility to LEN ranging from 4- to >3,226-fold relative to WT

- Most LEN-selected mutants displayed substantially reduced replicative fitness relative to the WT virus

- LEN-selected mutants retained WT susceptibility against representative drugs from the 4 main drug classes (NRTI, NNRTI, PI, INSTI) and MI

- CA protein was found to be ≥91% conserved across HIV-1 sequences (n=10,512 sequences) with 100% conservation at LEN-selected positions associated with >10-fold reduced susceptibility to LEN (L56I, M66I, K70N, N74D), indicating a low likelihood that LEN-resistance mutations may be pre-existing in people with HIV

- Irreversible inactivation of infectious cell-free HIV-1 (virucidal EC_{50} value of 25 nM)
- No antagonism when combined with representative drugs from each of the 4 main classes, whereas moderate to high synergy was observed
- LEN demonstrated no activity against other human viruses such as HCV, HRV, HBV, and RSV at drug exposures relevant to the activity of LEN against HIV-1
- Low potential for off-target effects at a 10 μM concentration (≥5,000-fold above the estimated free drug concentration at C_{max} at the SC clinically efficacious dose) against a panel of 87 molecular targets
- In a single SC dose safety pharmacology study in rats, no LEN-related adverse changes occurred in rat respiratory or CNS endpoints up to 100 mg/kg
- In dogs, no ECG or blood pressure changes were attributed to LEN after a single dose of 100 mg/kg or after repeated SC doses of 30 mg/kg
- The free C_{max} at the no-observed-(adverse)-effect levels (NO(A)ELs) for respiratory, CNS and cardiovascular endpoints were 1.4, 1.4 and 20-fold higher than the free C_{max} after the 6-month clinical oral loading and subcutaneous dosing regimen

In summary, LEN is a novel potent, selective, multi-stage inhibitor of HIV-1 capsid function with unprecedented antiviral activity, unique mechanism of action and overall favorable preclinical pharmacology profile that supports its further development as a novel antiretroviral agent for the treatment of HIV-1 infection.

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SECTION 2.6.3—PHARMACOLOGY TABULATED SUMMARY

LENACAPAVIR

Gilead Sciences, Inc.

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CONFIDENTIAL AND PROPRIETARY INFORMATION

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1. PHARMACOLOGY OVERVIEW

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No.
Primary Pharmacodynamics					
Antiviral Activity and Cytotoxicity of GS-6207 in MT-4 Cells	No	Human lymphoblastoid T-cell line MT-4, HIV-1 IIIb virus	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2018
Antiviral Activity and Cytotoxicity of GS‑6207 in Primary CD4+ T-Lymphocytes and Monocyte-Derived Macrophages	No	Primary human CD4+ T-lymphocytes, primary human monocyte-derived macrophages, human PBMCs, HIV-1 BaL virus	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2019
Antiviral Activity of GS-6207 Against HIV Clinical Isolates	No	Human PBMCs, HIV-1 92UG031, HIV-1 92UG037, HIV-1 89BZ_167, HIV-1 90US_873, HIV-1 91US001, HIV-1 91US004, HIV-1 96th_NP1538, HIV-1 BaL, HIV-1 JR-CSF, HIV-1 YU-2, HIV-1, 92BR025, HIV-1 98US_MSC5016, HIV-1 92UG001, HIV-1 98UG_57128, HIV-1 CMU02, HIV-1 CMU08, HIV-1 93BR020, HIV-1 JV1083, HIV-1 YBF30, HIV-1 BCF01, HIV-1 90TH_CM235, HIV-1 01CM008BBY, HIV-1 91DJ263, HIV-2 CBL‑20, and HIV-2 CDC310319 viruses	In Vitro	[redacted] USA	PC-200-2020

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No.
Inhibition of HIV-1 Early-Stage and Late-Stage Replication Steps in MT-2 Cells by GS-6207	No	Human embryonic kidney cell line HEK293T transfected with DNA plasmids encoding VSVG-pseudotyped HIV-1 NL4-3-deltaEnv-FFLuc reporter virus with and without capsid mutations. Target cells: Human lymphoblastoid T-cell line MT-2. Virus: VSVG-pseudotyped recombinant HIV-1 virus NL4-3-deltaEnv-FFLuc containing firefly luciferase reporter gene, with and without capsid mutations	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA.	PC-200-2021
Binding of GS-6207 to Recombinant HIV-1 Capsid Protein and Effects on In Vitro Capsid Assembly	No	Purified recombinant cross-linked WT and mutant capsid hexamer protein, expressed and isolated from E. coli. In vitro capsid assembly: Purified recombinant WT HIV-1 capsid protein, expressed and isolated from E. coli	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA; [redacted] USA	PC-200-2022
Inhibition of Proper Capsid Core Formation and HIV-1 Assembly by GS-6207	No	Human lymphoblastoid T-cell line chronically infected with HIV-1 IIIb virus (MT-2/IIIb), with infectivity analysis on human lymphoblastoid T-cell line CEM-NKR CCR5+Luc+. HIV-1 assembly defect: Human embryonic kidney cell line HEK293T transfected with DNA plasmids encoding VSVG-pseudotyped HIV-1 NL4-3-deltaEnv-FFLuc reporter virus with and without capsid mutations	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA; [redacted] USA	PC-200-2023

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No.
Effect of GS-6207 on the Early Stages of HIV-1 Replication Cycle in Target Cells	No	Human lymphoblastoid T-cell line CEM-NKR CCR5+Luc+, recombinant HIV-1 virus NL4-3-deltaEnv-FFLuc containing firefly luciferase reporter gene and pseudotyped with envelope proteins of distinct tropism. Human PBMCs, HIV-1 virus NL4-3-BlaM-Vpr containing co-packaged β-lactamase reporter enzyme. Human lymphoblastoid T-cell line MT-2, VSVG-pseudotyped recombinant HIV-1 virus NL4-3-deltaEnv-FFLuc containing firefly luciferase reporter gene	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2024
In Vitro HIV-1 Resistance Selection Studies with GS-6207	No	Human lymphoblastoid T-cell line MT-2, clonal HIV-1 HXB2D virus. Human PBMCs, HIV-1 BaL, HIV-1 92US657, HIV-1 91US006, HIV-1 7576, HIV-1 7406, HIV-1 7476	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2025
Characterization of Site-Directed HIV-1 Capsid Mutants Resistant to GS-6207	No	Human lymphoblastoid T-cell line MT-2, VSVG-pseudotyped recombinant HIV-1 virus NL4-3-deltaEnv-FFLuc containing firefly luciferase reporter gene. Human lymphoblastoid T-cell line CEM-NKR CCR5+Luc+, recombinant HIV-1 virus NL4-3-JRFL-secNLuc encoding secreted NanoLuc luciferase reporter gene, with and without various site-directed capsid mutations. Primary human CD4+ T-lymphocytes, primary human monocyte-derived macrophages, recombinant HIV-1 virus NL4-3-JRFL-secNLuc encoding secreted NanoLuc luciferase reporter gene, with and without various site-directed capsid mutations	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2026

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No.
Activity of GS-6207 Against Drug-Resistant HIV-1 Mutant Variants	No	Human lymphoblastoid T-cell line MT-2, HIV-1 HXB2D virus and HXB2D virus variants containing various site-directed mutations associated with NRTI, NNRTI, INSTI or PI resistance	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2027
Effects of Multiplicity of Infection (MOI) on In Vitro Antiviral Activity of GS-6207	No	Human lymphoblastoid T-cell line CEM-NKR CCR5+Luc+, recombinant HIV-1 virus NL4-3-JRFL-secNLuc encoding secreted NanoLuc luciferase reporter gene	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2031
Assessment of HIV-1 Capsid Protein Polymorphisms in the GS-6207 Binding Site	No	In silico analysis of viral DNA sequences from human patients at the population and deep sequence level	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA; [redacted] Germany	PC-200-2032
Antiviral Activity of GS-6207 Against a Panel of HIV-1 Patient Isolates	No	Human lymphoblastoid T-cell line MT-2, HIV-1 Patient Isolates	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2036
Antiviral Activity of GS-6207 Against HIV Gag Cleavage Site Mutations	No	Human lymphoblastoid T-cell line MT-2, HIV-1 Patient Isolates and Site-Directed Mutants	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2037
Impact of HIV-1 Capsid Protein Polymorphisms On GS-6207 Antiviral Activity	No	Human lymphoblastoid T-cell line MT-4, HIV-1 NL4.3 virus and Site-Directed Mutants	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2038
Antiviral Activity of Lenacapavir Against a Panel of HIV-1 Clinical Isolates With Diverse HIV Subtype	No	Human Embryonic Kidney (HEK293T) Cell Line, Patient-Derived Recombinant HIV-1 Isolates	In Vitro	[redacted] USA	PC-200-2041

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No.
Antiviral Activity of LEN In Isolates with Resistance to the Four Main Antiretroviral Classes	No	Human Embryonic Kidney (HEK293T) Cell Line, Patient-Derived Recombinant HIV-1 Isolates	In Vitro	[redacted] USA	PC-200-2044
Antiviral Activity of LEN In LEN-Resistant Site-Directed Mutants in the Replicative Multicycle Assay	No	Human lymphoblastoid T-cell line MT-2, HIV-1 Site-Directed Mutants	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2045
Antiviral Activity of LEN In LEN-Resistant Site-Directed Mutants in the Single-Cycle Assay	No	Human Embryonic Kidney (HEK293T) Cell Line, Site-Directed Mutant Recombinant HIV-1 Viruses	In Vitro	[redacted] USA	PC-200-2046
Secondary Pharmacodynamics					
Molecular Target Screen of GS-6207	No	Human, rat, hamster, rabbit, guinea pig, and pig receptors and transporters	In Vitro	[redacted] Taiwan,	PC-200-2012 ([redacted] AB35840)
Cytotoxicity of GS-6207 in Human Cell Lines and Primary Cells Non-permissive to HIV-1 Infection	No	Human hepatoma cell line Huh7, galactose-adapted human hepatoma cell line Gal-HepG2, galactose-adapted human prostate carcinoma cell line Gal-PC-3, normal human embryonic lung fibroblast cell line (MRC-5), and primary human hepatocytes	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2028

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No.
Antiviral Activity of GS-6207 Against Non-HIV Viruses	No	**HCV:** Human carcinoma cell line Huh7-derived HCV-1b (Con1/SG-hRLucNeo) and HCV-2a (JFH-1/hRLucNeo2a) replicons **HBV:** Human hepatoma cell line HepG2 stably expressing NTCP receptor (HepG2-NTCP), infected with HBV AD38 virus **HRV:** Human cervical adenocarcinoma cell line H1-HeLa, infected with HRV-16 virus **RSV:** Human epithelial type 2 cell line HEp-2, infected with RSV A2 virus	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2029
Safety Pharmacology					
Effect of GS-6207 on the Respiratory System of Rats	Yes	Rat/Crl:WI(Han)	SC	[redacted] USA	PC-200-2014 ([redacted] 8353957)
Effect of GS-6207 on the Central Nervous System of Rats	Yes	Rat/Crl:WI(Han)	SC	[redacted] USA	TX-200-2005 ([redacted] 8353952)
Effect of GS-6207 on the Cardiovascular System of the Dog	Yes	Dog/Beagle	SC	[redacted] USA	TX-200-2006 ([redacted] 8353953)

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No.
Pharmacodynamic Drug Interactions					
In Vitro Antiviral Activity of GS-6207 in Combination with Other Antiretroviral Agents	No	Human lymphoblastoid T-cell line MT-2, HIV-1 IIIb virus	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-200-2030

CRO = contract research organization; GLP = good laboratory practice; HBV = hepatitis B virus; HCV = hepatitis C virus; HRV = human rhinovirus; PBMC = peripheral blood mononuclear cell; PCR = polymerase chain reaction; RSV = respiratory syncytia virus; SC = Subcutaneous injection

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

2. PRIMARY PHARMACODYNAMICS

Test Article: LEN

Organ Systems Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro	Human lymphoblastoid T-cell line MT-4, HIV-1 IIIb virus	In Vitro	NA	NA	LEN displays sub-nanomolar potency and high selectivity index in MT4 cells	No	PC-200-2018
In vitro	Human PBMCs, CD4+ T-lymphocytes, monocyte-derived macrophages; HIV-1 BaL virus	In Vitro	NA	NA	LEN displays sub-nanomolar potency and high selectivity index in PBMCs	No	PC-200-2019
In vitro	Human PBMCs; HIV-1 and HIV-2 isolates	In Vitro	NA	NA	LEN displays sub-nanomolar potency in PBMCs against HIV-1 isolates representing major HIV-1 subtypes	No	PC-200-2020
In vitro	Human embryonic kidney cell line (HEK293T), DNA plasmids encoding VSVG-pseudotyped HIV-1 NL4-3-deltaEnv-FFLuc reporter virus with and without capsid mutations; Human lymphoblastoid T-cell line MT-2	In Vitro	NA	NA	LEN displays sub-nanomolar potency at both early and late stages of HIV replication	No	PC-200-2021
In vitro	Purified recombinant WT and mutant capsid monomer and hexamer protein expressed and isolated from E. coli	In Vitro	NA	NA	LEN binds directly to HIV capsid protein and dysregulates the kinetics of capsid assembly/disassembly	No	PC-200-2022

Test Article: LEN

Organ Systems Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro	Human lymphoblastoid T-cell line chronically infected with HIV-1 IIIb virus (MT-2/IIIb), human lymphoblastoid T-cell line CEM-NKR CCR5+Luc+, Human embryonic kidney cell line (HEK293T), DNA plasmids encoding VSVG-pseudotyped HIV-1 NL4-3-deltaEnv-FFLuc reporter virus with and without capsid mutations	In Vitro	NA	NA	HIV production in the presence of LEN led to non-infectious particles with irregularly shaped capsid cores	No	PC-200-2023
In vitro	Human lymphoblastoid T-cell line CEM-NKR CCR5+Luc+, recombinant HIV-1 virus NL4-3-deltaEnv-FFLuc containing firefly luciferase reporter gene and pseudotyped with envelope proteins of distinct tropism	In Vitro	NA	NA	LEN inhibits early steps in HIV infection between reverse transcription and integration	No	PC-200-2024
In vitro	Human lymphoblastoid T-cell line MT-2, human PBMCs; clonal HIV‑1 HXB2D virus, HIV-1 BaL and HIV-1 isolates	In Vitro	NA	NA	In vitro resistance selections with LEN identified 7 HIV-1 capsid mutations	No	PC-200-2025

Test Article: LEN

Organ Systems Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro	Human lymphoblastoid T-cell line MT-2, Human lymphoblastoid T-cell line CEM-NKR CCR5+Luc+, Primary human CD4+ T-lymphocytes and monocyte-derived macrophages, VSVG-pseudotyped recombinant HIV-1 virus NL4-3-deltaEnv-FFLuc, recombinant HIV-1 virus NL4-3-JRFL-secNLuc encoding secreted NanoLuc luciferase reporter gene, with and without various site-directed capsid mutations	In Vitro	NA	NA	LEN in vitro-selected resistance mutations confer a wide range of reduction in LEN susceptibility and are associated with a reduction in replication capacity	No	PC-200-2026
In vitro	Human lymphoblastoid T-cell line MT-2, HIV-1 HXB2D WT and variants containing various site-directed mutations associated with NRTI, NNRTI, INSTI or PI resistance	In Vitro	NA	NA	HIV-1 mutants with resistance to NRTIs, NNRTIs, PIs, and/or INSTIs remain fully susceptible to LEN in vitro	No	PC-200-2027

Test Article: LEN

Organ Systems Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro	Human lymphoblastoid T-cell line CEM-NKR CCR5+Luc+, recombinant HIV-1 virus NL4‑3-JRFL-secNLuc encoding secreted NanoLuc luciferase reporter gene	In Vitro	NA	NA	LEN in vitro susceptibility is affected by multiplicity of infection (MOI)	No	PC-200-2031
In vitro	In silico analysis of viral DNA sequences from human patients at the population and deep sequence level	In Vitro	NA	NA	The LEN binding site is highly conserved across a large panel of published sequences	No	PC-200-2032
In vitro	Human lymphoblastoid T-cell line MT-2, HIV-1 Patient Isolates	In Vitro	NA	NA	LEN in vitro potency is not affected by the presence of Gag polymorphisms in isolates from treatment-naïve or treatment-experienced people in contrast to that of maturation inhibitors	No	PC-200-2036
In vitro	Human lymphoblastoid T-cell line MT-2, HIV-1 Patient Isolates and Site-Directed Mutants	In Vitro	NA	NA	LEN in vitro potency is not affected by the presence of Gag cleavage site mutations	No	PC-200-2037

Test Article: LEN

Organ Systems Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro	Human lymphoblastoid T-cell line MT-4, HIV-1 NL4.3 virus and Site-Directed Mutants	In Vitro	NA	NA	CA polymorphisms at the LEN binding site have a low likelihood to cause phenotypic resistance to LEN	No	PC-200-2038
In vitro	Human Embryonic Kidney (HEK293T) Cell Line, Patient-Derived Recombinant HIV-1 Isolates	In Vitro	NA	NA	LEN displays sub-nanomolar potency in a 2nd panel of HIV-1 isolates representing major HIV-1 subtypes	No	PC-200-2041
In vitro	Human Embryonic Kidney (HEK293T) Cell Line, Patient-Derived Recombinant HIV-1 Isolates	In Vitro	NA	NA	LEN in vitro susceptibility is maintained against a panel of patient isolates with resistance to NRTIs, NNRTIs, PIs, and/or INSTIs	No	PC-200-2044
In vitro	Human lymphoblastoid T-cell line MT-2, HIV-1 Site-Directed Mutants	In Vitro	NA	NA	Mutants harboring LEN in vitro-selected resistance mutations remain susceptible to PIs and MIs in multicycle phenotypic assay	No	PC-200-2045
In vitro	Human Embryonic Kidney (HEK293T) Cell Line, Site-Directed Mutant Recombinant HIV-1 Viruses	In Vitro	NA	NA	Mutants harboring LEN in vitro-selected resistance mutations remain susceptible to PIs in single-cycle phenotypic assay	No	PC-200-2046

CRO = contract research organization; GLP = good laboratory practice; HBV = hepatitis B virus; HCV = hepatitis C virus; HRV = human rhinovirus; INSTI = integrase strand-transfer inhibitor; MI = maturation inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PBMC = peripheral blood mononuclear cell; PCR = polymerase chain reaction; PI = protease inhibitor; RSV = respiratory syncytia virus; SC = Subcutaneous
a An entry of "Yes" indicates that the study includes a GLP compliance statement.

Studies of the primary pharmacodynamics of LEN are presented in Section 2 of Module 2.6.2.

3. SECONDARY PHARMACODYNAMICS

Test Article: LEN

Organ Systems Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro	Human, rat, hamster, rabbit, guinea pig, and pig receptors and transporters	In Vitro	NA	NA	LEN showed low potential for off-target effect against a panel of 87 molecular targets tested at high LEN concentration (10 µM)	No	PC-200-2012
In vitro	Human hepatoma cell line Huh7, galactose-adapted human hepatoma cell line Gal-HepG2, galactose-adapted human prostate carcinoma cell line Gal-PC-3, normal human embryonic lung fibroblast cell line (MRC-5), and primary human hepatocytes	In Vitro	NA	NA	LEN did not display significant cytotoxicity against 4 cell lines (Huh7, HepG2, PC-3, MRC-5) and primary hepatocytes	No	PC-200-2028

Test Article: LEN

Organ Systems Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro	Human carcinoma cell line Huh7-derived HCV-1b and HCV-2a replicons; human hepatoma cell line HepG2 (HepG2-NTCP) infected with HBV AD38 virus; human cervical adenocarcinoma cell line H1-HeLa infected with HRV-16 virus; human epithelial type 2 cell line HEp-2 infected with RSV A2 virus	In Vitro	NA	NA	LEN did not exert antiviral activity against HCV, HRV, RSV, and HBV at drug exposures relevant for HIV inhibition	No	PC-200-2029

CRO = contract research organization; GLP = good laboratory practice; HBV = hepatitis B virus; HCV = hepatitis C virus; HRV = human rhinovirus; PBMC = peripheral blood mononuclear cell; PCR = polymerase chain reaction; RSV = respiratory syncytia virus; SC = Subcutaneous
a An entry of "Yes" indicates that the study includes a GLP compliance statement.

Studies of the secondary pharmacodynamics of LEN are presented in Section 3 of Module 2.6.2.

4. SAFETY PHARMACOLOGY

4.1. In Vitro Studies

No in vitro safety pharmacology studies were conducted.

4.2. In Vivo Studies

Test Article: LEN

Organ Systems Evaluated	Species / Strain	Method of Administration	LEN Dose[a] (mg/kg/dose)	Gender and n per Group	Noteworthy Findings	GLP[b]	Gilead Study No. (CRO Study No.)
Central Nervous System	Rat/Crl:WI(Han)	SC - 4 Doses (Every other week)	0[c], 10, 30, 100	M: 10	None NOEL = 100 mg/kg	Yes	TX-200-2005 ([redacted] 8353952)
Respiratory System	Rat/Crl:WI(Han)	SC	0[c], 10, 30, 100	M: 8	100 mg/kg: minor non-statistically significant increase in tidal volume and a decrease in respiration rate NOEL = 30 mg/kg	Yes	PC-200-2014 ([redacted] 8353957)
Cardiovascular System	Dog/Beagle	SC - 4 Doses (Every other week)	0[c], 10, 30, 100	M:6/F:6[a]	None NOEL Single dose = 100 mg/kg NOEL Repeat dose = 30 mg/kg	Yes	TX-200-2006 ([redacted] 8353953)

NOEL = No Observed Effect Level; M = Male; F = Female; SC = Subcutaneous
a In the in vivo cardiovascular study 4 SC doses were administered to 6M, 6F for 0, 10, 30 mg/kg; a single dose of 100 mg/kg SC was administered to 6M and 2F.
b An entry of "Yes" indicates that the study includes a GLP compliance statement.
c Vehicle control article was 2% [w/v] poloxamer 188 prepared in 0.9% [w/v] sodium chloride, in sterile water for injection.

Studies of the in vivo safety pharmacology of LEN are presented in Section 4 of Module 2.6.2.

5. PHARMACODYNAMIC DRUG INTERACTIONS

Test Article: LEN

Organ Systems Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro	Human lymphoblastoid T-cell line MT-2, HIV‑1 IIIb virus	In Vitro	NA	NA	LEN showed synergy and no antagonism when combined pairwise with representative drugs from the 4 main ARV drug classes	No	PC-200-2030

ARV = antiretroviral; CRO = contract research organization; GLP = good laboratory practice; HBV = hepatitis B virus; HCV = hepatitis C virus; HRV = human rhinovirus; PBMC = peripheral blood mononuclear cell; PCR = polymerase chain reaction; RSV = respiratory syncytia virus; SC = Subcutaneous
a An entry of "Yes" indicates that the study includes a GLP compliance statement.

Studies of the pharmacodynamics drug interactions of LEN are presented in Section 3 of Module 2.6.2.